

## Research article

## Morphological, biochemical and DNA barcoding characteristics for some *Lantana* L. cultivars growing in Egypt

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**Abstract:** *Lantana* cultivars are aromatic shrubs or herbs cultivated for ornamental purposes for its different flower colors in addition to its use as hedges in Egypt. The similarity of the morphological characteristics between *Lantana camara* and *L. depressa* varieties lead to carry out the present study where five varieties cultivated in Helwan University Garden, Egypt of red, dark purple, pale yellow, dark yellow and mixed colors flowers varieties has been done on the bases of morphological, chemical study to determine total antioxidant compounds, total phenolics and flavonoids, total anthocyanin content, total carotenoids content, total proteins, and nucleic acids contents for the flowers of each cultivar, in addition to the molecular levels on the bases of DNA barcoding level where the Internal Transcribed Spacer (ITS) discriminate and confirm the identification of four *Lantana* ornamental plant varieties at the variety level.

**Keywords:** Ornamentals - Antioxidant - Phenolics - Anthocyanins - Carotenoids - Nucleic acids.

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### INTRODUCTION

*Lantana* species are found in different tropical and subtropical environments. Most of *Lantana* plants are either Spanish flag (species of section *Lantana* and their hybrids, including *Lantana camara* L., *L. depressa* Small, *L. hirsuta* Martens & Galeotti, *L. horrida* Kunth, *L. splendens* Medik., *L. x strigocamara* R.W.Sanders, etc.), or trailing *Lantana* [*L. montevidensis* (Spreng.) Briq.] Baldwin (1995). Numerous cultivars of the Spanish flag exist; including 'Irene', 'Christine' and 'Dallas Red' (all tall-growing cultivars) and several recently introduced shorter ones. The shorter cultivars may flower more regularly than the taller cultivars for example, *Lantana montevidensis* gives blue or white flowers all year round.

*Lantana* cultivars cultivated for ornamental purpose where leaves have medicinal value; used for snake bites and treatment of tetanus disease, in addition to its use as hedges in Egypt as well as in Kenya, Tanzania and Uganda (Soliman & Amer 2002). Although *Lantana* cultivars are generally being somewhat toxic, usually rejected by herbivores, they may still become infested with pests.

The edibility of *Lantana* berries is contested. *Lantana* berries are edible when ripe (Boulos 2002, Bell *et al.* 2007, Borsch & Quandt 2009), though like many fruits are mildly poisonous if eaten while still green. Some scientists indicated from their experimental investigations that both undeveloped and developed *Lantana* fruits are actually fatal, while others claimed that the developed ones are not fatal (CBOL Plant Working Group 2009). Extracts of *Lantana camara* may be used for protection of cabbage against the aphide (Calonje *et al.* 2009). The essential oils of *L. camara* and *L. montevidensis* are used as a source of plant-derived natural products with resistance-modifying antibacterial activity (Erlânio *et al.* 2012). Also, *Lantana* can be used to produce furniture which is resistant to sun, rain, and termite damage as an alternative to highly priced cane and bamboo in Soliga, Korava and Palliyar in southern Karnataka, India (Chase *et al.* 2005).

*Lantana* cultivars are aromatic shrubs 0.4–2.0 m or herbs; stems carbid-hairy or prickly; leaves opposite, 2–8 × 2–5 cm, ovate, toothed, acute, crenate-serrate, the base subcordate; petiole 0.8–1.8 cm; glandular-punctate; flowers in axillary heads 2.5–4.0 cm diameter; peduncle 1.5–6.0 cm; bracts 4–8 × 1–2 mm; sessile, in axillary pedunculate heads or spike-like inflorescences; flowers bracteates; calyx 3–4 mm, membranous, truncate or

sinate-dentate; corolla 0.8–1.2 cm, red, yellow, purple, orange or whitish; corolla-tube densely puberulent outside; limb obscurely 2–8 mm, 4–5 lobed; stamens 4, didynamous, included; ovary 2-locular, each locule with 1 ovule; style short; stigma thick, oblique; fruit a fleshy drupe separating into two 1-locular pyrenes. The fruit of *L. camara* is a berry-like drupe which turns from green to dark purple when mature. Both vegetative (asexual) and seed reproduction occur. The spreading of *Lantana camara* seeds which are produced by the individual plant (nearly 12,000 fruit per plant) was facilitated by fowls and other creatures.

*Lantana* L. species found mainly in tropical America, few tropical and South Africa (Sharma 1981, Boulos 2002). In Egypt, *Lantana* L. has been classified as *L. camara* and *L. viburnoides* (Forssk.) Vahl by Boulos (2002), both have the same morphological characters except that the stems of *L. camara* covered with recurved prickles and inflorescence not elongated after anthesis.

Owing to wide discriminating breeding throughout the Centuries (17<sup>th</sup> and 18<sup>th</sup>) to use as a decorative plant, there are now diverse *L. camara* cultivars. *Lantana* L. sub-classification depending on morphological character may be not successful. Accordingly, depending on the secondary products in plants, such as flavonoids and anthocyanin pigment, are abundant and are suitable as biochemistry indicator in chemotaxonomy (Harborne 1967). Additionally, the phenolic components of plants can be valuable in sub-classification of plants. Polyphenols have been used in the chemotaxonomy of various crops like *Sorghum bicolor* L. (Dicko *et al.* 2002); *Galanthus caucasicus* (Baker) Grossh., *Magnolia obovata* Thunb., *Cocculus laurifolius* DC., *Veratrum lobelianum* Bernh. (Tsakadze *et al.* 2005) and *Theobroma cacao* L. (Niemenak *et al.* 2006). Moreover, anthocyanins was used in chemotaxonomy to place *Crocus* species and cultivars into seven chemotypes (Nørnbæk *et al.* 2002).

Carotenoids are yellow, orange, and red pigments that are widely distributed in nature, it play an important role in photosynthesis and furnishing flowers and fruits with distinct colors. While the carotenoid composition of flower petals varies from species to species. List of carotenoid composition in flower petals and discussion for the possible causes of flowers qualitative diversity has been reported by Ohmiya (2011). In addition, the qualitative composition and localization of carotenoids in leaflets of ten species of the genus *Ceratozamia* (Cycads) was used to differentiate between them (Cardini & Morassi 2005).

Recently, DNA barcoding is a molecular tool that uses a short locus from a standardized genome position to provide fast and accurate species identification since their first application by (Porter & Collins 1991, Kress & Erickson 2007). ITS1 and ITS2 are widely used for phylogeny reconstruction, (Baldwin *et al.* 1995, Liston *et al.* 1996) and it is suitable for evolutionary studies at the species or generic level, Poczai & Hyvonen (2010). This technique is helpful in taxonomic, ecological, and evolutionary as stated by Lahaye *et al.* (2008) and Ragupathy *et al.* (2009). Although, the most important characteristic features of a DNA barcode are its universality, specificity on variation and easiness on employment. Several different loci, and combinations of there have been suggested as suitable barcodes for land plants (Pennisi 2007, Ford *et al.* 2009). For instance, the nuclear internal transcribed spacer (ITS) region and the plastid intragenic spacer trnH-psbA have been proposed for flowering plants (Kress *et al.* 2005).

Internal Transcribed Spacer (ITS) is one of the most used polymorphic regions is, a space of non-coding RNA situated between structural ribosomal RNAs on a common precursor transcript. ITS spacer is known to be partitioned into ITS1 and ITS2 separated by 5.8S ribosomal cistron, in which the RNA poly-cistronic precursor transcript will be in this order 18S rRNA, ITS1, 5.8S rRNA, ITS2, 26S (Wheeler & Honeycutt 1988). As a part of the transcriptional unit of rDNA, the ITS spacers 1 and 2 are therefore present in all organisms (Calonje *et al.* 2009). The aim of this work is to discriminate and confirm the identification for four of five *Lantana* ornamental plants at the molecular level, using ITS regions in addition to some chemical characteristics of the five plant varieties. The present work aimed to link different *Lantana* species by their chemical analysis for *Lantana* flower-corolla- may which show certain lines between these different species. Then confirm their morphological identification through reference sequences in database and GeneBank.

## MATERIALS AND METHODS

### *Samples collection*

Five samples were collected from Helwan Univ. campus Garden, Cairo, Egypt; have been identified according to the inflorescence color petals; (1) Multicolor, (2) Pale yellow, (3) Deep purple, (4) Ded and (5) Deep yellow flowers. About fifty flowers for each specimens for determining and characters description.

### *Total phenolics and flavonoids content*

Extraction was carried out according to Stanković (2011). Then the extract was used for the determination of

their content.

Total phenolic compounds content was determined by the method of Savitree *et al.* (2004). 0.5 ml of each extract of samples was mixed with 2.5 ml of Folin-Ciocalteu reagent (10 folds diluted) and 2.0 ml of 7.5% sodium carbonate, then reading the absorbance at 760 nm after incubation for 30 minutes at room temperature. The total phenol content of the samples was calculated as mg g<sup>-1</sup> d. wt. using the standard curve of gallic acid.

Total flavonoids content was estimated according to Zhishen *et al.* (1999) method, flowers extract (1.0 ml) was mixed with 0.30 ml NaNO<sub>2</sub> solution (10%) and the total volume completed to 5.3 ml with dis. water. 0.30 ml AlCl<sub>3</sub> solution (10%) and 2.0 ml 1% NaOH solution were added after incubation period. The absorbance was measured at 510 nm versus the blank. The total flavonoids content of the samples was calculated as mg/g<sup>-1</sup>d.wt using the standard curve of quercetin (0–12 mg ml<sup>-1</sup>).

#### *Nucleic acids content*

Nucleic acids content was carried out by Marmur (1961) method for extraction and Dische & Schwartz (1973) method for DNA determination. The plant extract (1 ml) and 2 ml of diphenylamine solution were incubated for 10 minutes at 100°C. The absorption was measured at 595 nm and DNA was calculated as mg g<sup>-1</sup> F. Wt. by using DNA calibration curve. RNA was determined using the method of Ashwell (1957). The flower corolla extract (3 ml), 3 ml of FeCl<sub>3</sub> reagent and 0.3 ml orcinol solution were incubated in a boiling water bath for 40 minutes. The absorbance was measured at 670 nm and RNA expressed as mg g<sup>-1</sup> F.Wt. by using RNA calibration curve.

#### *Total soluble proteins content*

Total soluble proteins content was determined according to Lowry *et al.* (1951) method. A sample of the extract was mixed with 1 ml freshly prepared solution of 2% sodium carbonate in 4% sodium hydroxide and 0.5% copper sulphate in 1% sodium tartrate. Folin reagent (0.1 ml) was added after 10 minutes of incubation and the optical density of the mixture was measured after 30 minutes at 700 nm. Total protein content was calculated as mg g<sup>-1</sup> d.wt. using calibration curve of albumin.

#### *Total Anthocyanin Content*

Total Anthocyanin Content was determined according to Hodges & Nozzolillo (1996) method. Fresh flowers petals were crushed in (2 ml) acidified methanol in a mortar. Centrifuge and incubate sample in a water bath at 50°C for one hour. The absorbance was measured at 540 nm and 600 nm. The amount of total anthocyanin contents was calculated in the original sample at 520 nm and 600 nm, expressed as mg l<sup>-1</sup>.

#### *TLC separation of Anthocyanin*

TLC separation of Anthocyanin was carried out according to Simona *et al.* (2008) method. Pigments were extracted from the flower petals of different color flowers by using 5 ml ethanol to homogenize 1 g of the petals tissue. Then the homogenate was heated for few minutes, and 100 µl of each extract was applied on the silica gel plates of 20 × 20 cm, Merck. The used running solvent mixture was contained: 1-butanol: acetic acid: water (4:1:5, v/v/v). The R<sub>f</sub> value for each band was then calculated.

#### *Total Carotenoids Content*

Total carotenoids Content was estimated following Metzener *et al.* (1965) method amended by Lichtenthaler (1987). Fresh leaves was homogenized with 85% acetone and centrifuged. Then the supernatant was measured at 3 wave lengths 452 nm, 645 nm, 664 nm. The total carotenoids content was expressed as µg g<sup>-1</sup> fresh weight of tissue. The concentration of carotenoids was calculated according to the following equations:

$$\text{Chlorophyll } a = 10.3 E_{664} - 0.918 E_{645}$$

$$\text{Chlorophyll } b = 19.7 E_{645} - 3.87 E_{664}$$

$$\text{Carotenoids} = 4.3 E_{452} (0.0265 \text{ Chl.}a + 0.426 \text{ Chl.}b)$$

#### *Molecular DNA extraction*

Molecular DNA extraction of Floral plant petal samples for the five *Lantana* varieties; Multicolor, pale yellow, dark purple, red and dark yellow was carried out using SIGMA® Plant High Molecular DNA extraction KIT®, Plant tissue was disrupted by grinding in liquid nitrogen and DNA was released with detergent and chaotropic agents.

After 10 minute precipitation and centrifugation by filtration column, cell debris with polysaccharides and proteins were removed. Then, microcentrifuge spin columns used for DNA purification.

DNA quality was tested using agarose gel electrophoresis, visualized by pre-added RedSafe® (5 µl per 100 ml) under UV light and quantified using Eppendorf® Spectrophotometer X100 device, about 50 µg of DNA

were obtained from 2 g ground powder of dry plant material. PCR and sequencing Two primers, ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TATTGA TAT GC-3') were used to amplify the internal transcribed spacer (ITS) according to White *et al.* (1990). PCRs of 50 ul reaction mixture (1X Flexi buffer, 50ng DNA template, 2.5 mM MgCl<sub>2</sub>, 10 μM dNTPs, 0.4uM of each primer, and 1U Promega© Green Go Taq™ enzyme) were performed, standard PCR profile with 55°C annealing temperature was used to amplify ITS. Results were tested on 1.5% agarose gel electrophoresis and visualized by pre-added 1x RedSafe® using a UV light. When successful, amplified fragments were cleaned and concentrated using Thermo Gene JET PCR Purification Kit #K0702. Cleaned fragments were sequenced by private service (Macrogen, Netherlands). Sequence chromatograms were compiled using Bioedit V3 to assemble the sequences. All sequences were manually aligned, while gaps inserted to preserve nucleotide homology. Ambiguous regions were deleted from the analyses. All Haplotype sequences were submitted into the GeneBank database (<http://www.ncbi.nlm.nih.gov>; accessions KR998497– KP998498 for ITS).

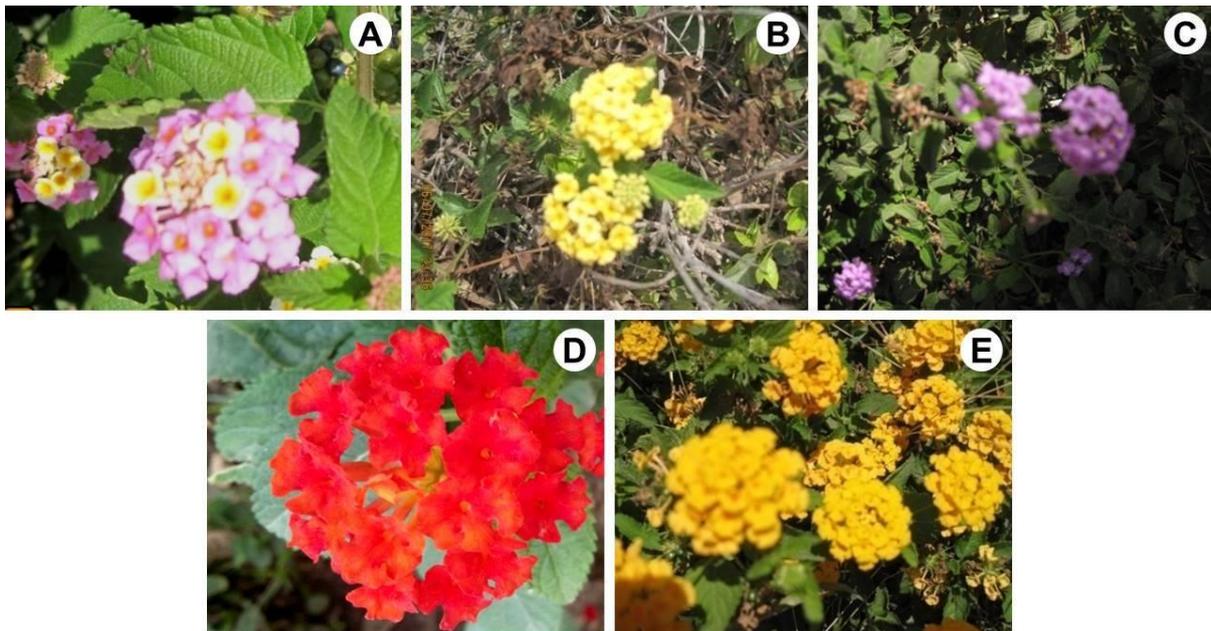
To identify the evaluative position and study phylogenetic relationships of the four varieties, the aligned sequences were analyzed by maximum likelihood (ML) analysis implemented in MEGA6 (Tamura *et al.* 2013). Tree inference options were set to Nearest Neighbor Interchange. Gaps/missing data were treated as partial deletions with site coverage cut off 95%. A bootstrap analysis with 1000 replicates was carried out in order to study the clade support values. The available ITS sequencing by BLASTn tool (NCBI) generate the trees in all methods. Maximum Composite Likelihood model was followed in our analysis. The rate variation among sites was modeled with a gamma distribution (Shape parameter = 0.48). The consensus tree was obtained after bootstrap analysis, with 1,000 replications, with values above 50% was reported. The analysis involved 22 and 26 nucleotide sequences for ITS.

## RESULTS

**Morphological identification:** Based on the morphological aspect, the main morphological characters have been represented in (Table 1; Fig. 1). **The phenolics and flavonoids content:** The present results indicated that the red flowers contain the premier content of phenolics and flavonoids followed by the deep yellow petals. Further, deep purple had the lowest content of phenolics and flavonoids (Table 2; Fig. 2). The high phenolics and flavonoids content of red flowers make a distinction of them from other species.

**Table 1.** Morphological characters for *Lantana* taxa studied of different inflorescences colors.

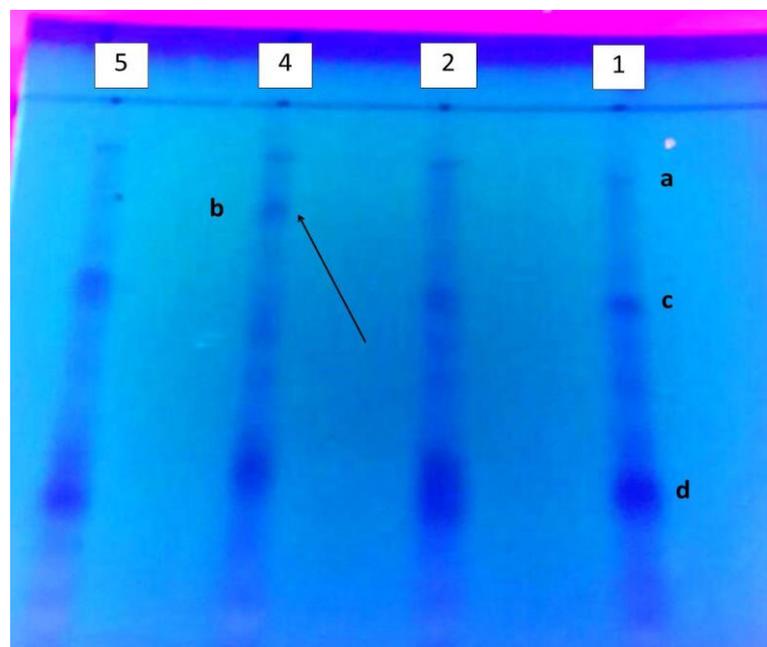
Character		Multi Colour	Pale Yellow	Deep Yellow	Deep Purple	Red
<b>Habit</b>		Shrub	Herb	Herb	Herb	Shrub
<b>Stem</b>	Width (cm)	0.5	0.35	0.4	0.3	0.5
	Surface	Prickly	Smooth	Smooth	smooth	Rough
	Shape	Square	Square	Square	Square	Square
	Habitat	Erect stem	Weak stem	Weak stem	Weak stem	Erect stem
<b>Leaves</b>	Blade Max width (cm)	4.5	1.6	2.5	1.8	3.6
	Blade Max long (cm)	6.0	3.0	3.3	3.0	7.0
	Petiole length (cm)	1.5	0.5	0.8	0.3	1.3
		Decussate	Decussate	Decussate	Decussate	Decussate
	Texture	Rough	Smooth	Rough	Smooth	Rough
	Blade	Large	Small	Small	Small	Large
	position	Opposite	Opposite	Opposite	Opposite	Opposite
	Shape	Ovate	Lancelate to ovate	Lancelate	Ovate	Ovate
	Margin	Serrate	Serrate	Serrate	Crenate	Coronate
	Apex	Acuminate	Acute	Acute	Acute	Acuminate, acute
Color	Dark green	Pale green	Pale green	Pale green	Pale green	
<b>Inflorescence</b>	Number of flowers/ Infl.	36	26	30	20	36
	Fruit	Berry	Berry	Berry	Berry	Berry
	Peduncle	Corium	Corium	Long raches	Corium	Corium
	Peduncle length (cm)	4.8	3.8	2.8	6.2	3.6



**Figure 1.** The collected samples of different colors: A, Multicolor; B, Pale yellow; C, Deep purple; D, Red; E, Dark yellow samples respectively.

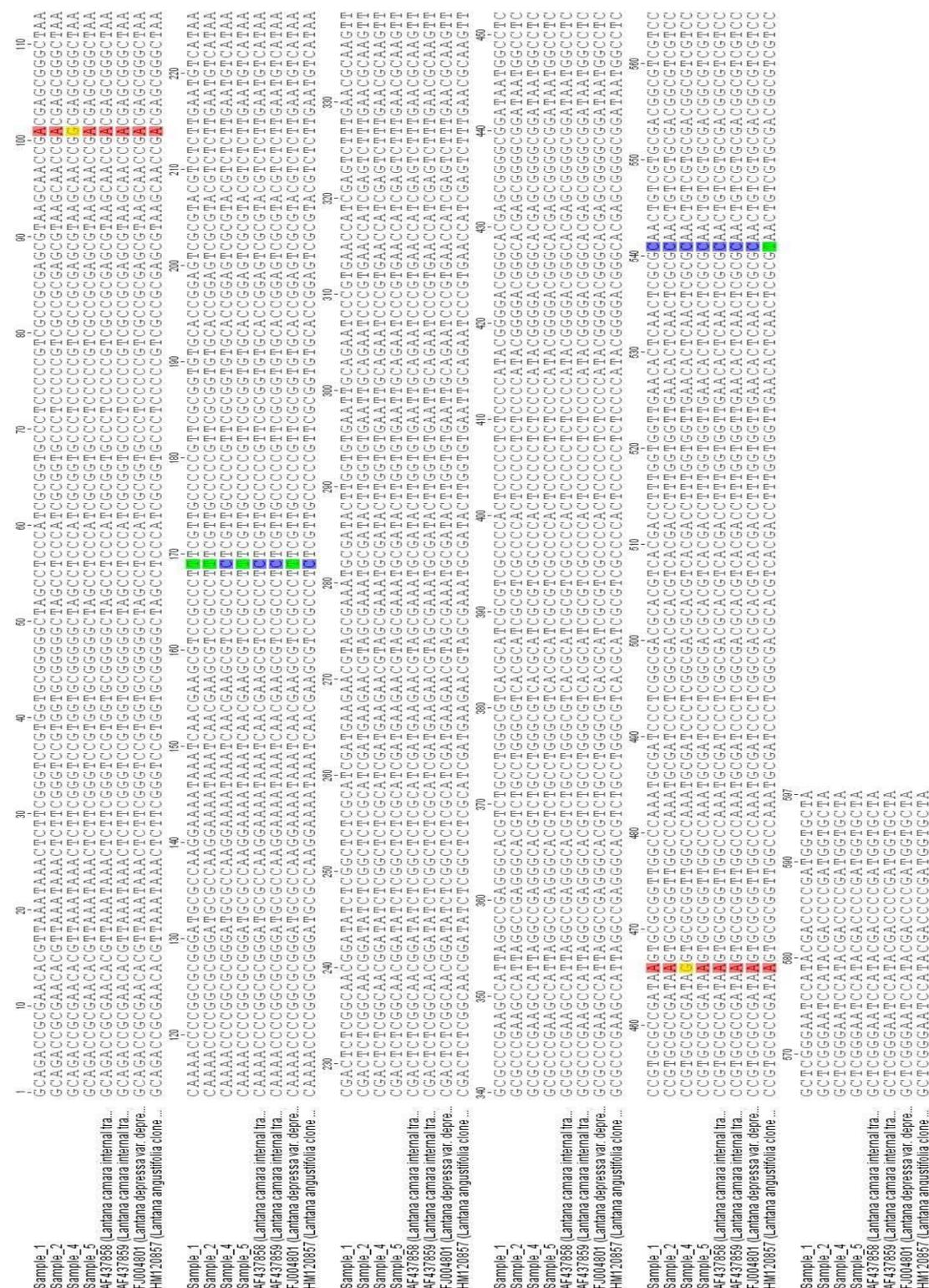
**Table 2.** Biochemical analysis for different colors inflorescences of *Lantana* taxa studied.

Biochemical character	Multi Colour	Pale Yellow	Deep Yellow	Deep Purple	Red
Anthocyanin content (mg g <sup>-1</sup> f.wt.)	16.7 ± 0.5	14.0 ± 0.06	16.1 ± 0.07	27.4 ± 1.0	37.0 ± 0.06
Carotenoids content (µg g <sup>-1</sup> f. wt.)	3.58 ± 0.07	11.93 ± 0.05	13.33 ± 0.15	1.03 ± 0.05	2.03 ± 0.02
Total phenolic compounds	93.25 ± 4.5	77.97 ± 0.83	102.8 ± 3.6	46.01 ± 0.62	126.7 ± 3.0
Total flavonoids	4.14 ± 0.16	4.90 ± 0.23	6.32 ± 0.62	1.54 ± 0.24	7.88 ± 0.29
DNA content (mg g <sup>-1</sup> f.wt.)	19.8 ± 0.26	11.7 ± 0.52	14.6 ± 0.25	15.7 ± 0.95	20.1 ± 0.14
RNA content (mg g <sup>-1</sup> f.wt.)	23.6 ± 0.10	40.5 ± 1.0	39.5 ± 0.17	36.8 ± 0.0	34.3 ± 0.17
Total Protein (mg g <sup>-1</sup> d.wt.)	83.37 ± 2.1	176.0 ± 1.8	204.1 ± 1.1	154.1 ± 4.7	145.8 ± 2.7



**Figure 2.** TLC of anthocyanin pigment in 1. Multicolor, 2. Pale yellow, 4. Red 5 deep yellow colors of *lantana* corolla flowers (showing characteristic arrowed band in red colored *lantana* flower).

The anthocyanin and total carotenoids content: The present study showed that, the petals of red flowers showed the highest content of anthocyanin. The anthocyanin content of red flowers was more than 2-fold of other flowers (pale yellow, deep yellow and mixed colors). In contrast, the red flowers showed the lowest content of the carotenoids from the other species and the yellow corolla shown the highest carotenoids content (Table 2; Fig. 3).



**Figure 3.** rbcl based maximum likelihood for collected samples of different *Lantana* specimens distinguished, Sample 1, 2, 4 and 5 representing Multicolor, pale yellow, red and dark yellow respectively.

The TLC separation of anthocyanin: The present study showed in the TLC chromatogram of red *Lantana* petals, the presence of 3(a, b and d) spots. One of them appeared only in the red *Lantana* (b band, Rf = 16). In the case of pale yellow, deep yellow and multicolor petals of *Lantana*, the chromatogram showed 3 spots (a, c and d).

The nucleic acids content: The nucleic acid extract showed dissimilar values of RNA content in the order of pale yellow > deep yellow > red > mixed colors (Table 2; Fig. 4). These results showed a relationship between the pale yellow and the deep yellow flowers of various *Lantana* varieties. The results revealed the maximum DNA value for the red flower (20.1) which powerfully related to mixed color flowers (19.8). The DNA content values showed the following order dark yellow > pale yellow> deep purple.



File: Mix\_B11\_premix.ab1 Run Ended: 2017/8/24 5:14:25 Signal G:1264 A:1974 C:4174 T:2516  
 Sample: Mix\_B11\_premix Lane: 93 Base spacing: 15.454962 720 bases in 8706 scans Page 1 of 2



**Figure 4.** DNA Barcoding graph for *Lantana* samples 1. multicolor, 2. Pale yellow, 3. Deep purple, 4. red and 5. deep yellow.

The total protein content: The protein content values in table (2) were ordered in the following deep yellow > pale yellow > red > multi-colors flowers. The results indicated that the yellow corolla contained the maximum content of proteins. The red corolla protein content value fallen in-between the yellow and the multi-colors ones.

Molecular Identification and DNA Barcoding: Using both regions (ITS), the phylogenetic analysis was performed using four off five samples together with GeneBank accessions (sample 1 ‘multicolor’, sample 2 ‘pale yellow’, sample 3 ‘deep purple’, sample 4 ‘red’ and sample 5 dark yellow flowers respectively). The trees was rooted between these samples that revealed two main clads, the first clade possessed three samples (multicolor, pale yellow and dark yellow varieties) belong to *Lantana depressa*, while the second clade of ITS phylogenetic analysis, showed that, sample 4 ‘red variety’ does belong to the same species, and not specifically to *Lantana depressa* nor to *Lantana camara*.

## DISCUSSION

Species of the genus *Lantana*, belonging to the family Verbenaceae. They have been of great interest for their ornamental, phytochemical, biological and pharmacological studies. The different varieties of *Lantana* have been reported to contain different types and levels of phytochemicals.

The maximum content of total phenolic, flavonoid compounds and anthocyanin of the red flower petals provides significant evidence for the distinction of this species from others. The flavonoid pathway has a pleiotropic role in plants, and one must consider that a single deviation in the pathway may have multiple phenotypic effects, Mary & Michael (2000). So there is an obstacle to link the phenotype to molecular changes.

Now, the genes of the flavonoid pathway are known, so it is essential to associate a phenotype to the gene family member responsible for flavonoid biosynthesis pathway. The unique pathway of the flavonoid biosynthesis in this species made it dissimilar from others.

Also, the highest content of DNA and anthocyanin pigment in the red flower petals confirmed the previous revealing. The water-soluble flavonoid pigments represent a class of phenylpropanoid secondary plant metabolites. The most important classes with regard to flower color are anthocyanins, flavonols and flavones. Flavonols and flavones as accessory pigments turned into anthocyanins giving the flowers their distinct colors (Goto 1987) and possess antioxidant properties (Stintzing *et al.* 2002, Einbond *et al.* 2004).

In the present work, the TLC analysis showed special band in the pigment extract of red flower with Rf 16 reflects the presence of cyaniding 3 glucoside, as a component of anthocyanin, Harborne (1967). While the malvidin 3 glucoside, Harborne (1967) with its band Rf 26 was observed in pale yellow, deep yellow and multi-color flowers and did not appear in red flower extract. The band with 50 Rf was observed in all extracts. This established another report of the correlation between the different species.

The biochemistry analysis of different colored flowers shows that; Flower color varied according to the different chemical composition of scent where In Lantana, the red flower variety exhibited significant differences between other varieties, while the multicolor and deep yellow flower varieties showed a certain association. The red flower variety was strongly associated to higher pheolics, flavonoids, anthocyanin pigment and DNA content than the other studied varieties (with other flower colors) were strongly associated with terpenoid compounds. This reflected the conserved biosynthetic pathways and the genetics of each variety which may have a critical role in determining specific associations between floral color and floral constituents.

The molecular identification and DNA Barcoding results show the first clade, possessed three samples 1, 3 and 5 (multi-color, pale yellow and deep yellow varieties) belong to *Lantana depressa*, while the second clade of ITS phylogenetic analysis, showed sample 4 of red variety does belong to the same species, and not specifically to *Lantana depressa* nor *Lantana camara*. This result was confirmed with the biochemistry analysis (of the present study) of the varieties of *Lantana* where red flower variety has been separated from other varieties (*camara* which included multicolor, pale yellow and deep yellow colored flowers) by their biochemical structure. In spite of (Kress *et al.* 2005, Chase *et al.* 2005) conclusion that the nuclear ITS region found to have a better resolution toward species and its varieties identification, The present result might be due to the insufficient similar sequences of these species in the GeneBank database. Even though the ITS was more efficient, it cannot be relied on as a single DNA barcoding region due to its variation within a single species (e.g. *Lantana camara*) or due to the presence of paralogs, orthologs and pseudogenes of ITS sequence in a single genome as suggested by (Feliner & Rosello 2007, Soltis *et al.* 2008), or due to the growing of *Lantana camara* wild among *Lantana depressa* populations, Hammer (2014).

## CONCLUSION

The present study provides preliminary assessment data that will be useful for wider application of DNA barcoding for ornamental plants. With the current development of primers, ITS will be very useful in barcoding for the ornamental plant species, where it has a better resolution toward species and varieties identification than the morphological. DNA barcoding together with the biochemical analysis provided a beneficial tool to sub-classify of different varieties of *Lantana L.*

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## REFERENCES

- Ashwell G (1957) *Methods in Enzymology III*. Inter-Science Publishers, Inc. New York.
- Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS & Donoghue MJ (1995) The ITS region of nuclear ribosomal DNA a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.
- Bell NE, Quandt D, ÓBrien TJ & Newton AE (2007) Taxonomy and phylogeny in the earliest diverging pleurocarps. square holes and bifurcating pegs. *The Bryologist* 110: 533–560.
- Borsch T & Quandt D (2009) Mutational dynamics and phylogenetic utility of noncoding chloroplast DNA. *Plant Systematics and Evolution* 282: 169–199.
- Boulos L (2002) *Flora of Egypt, Volume III*. Al-Hadara Publication, pp. 1–2.

- Calonje M, Martín-Bravo S, Dobes C, Gong W, Jordan- Thaden I, Kiefer C, Kiefer M, Paule J, Schmickl R & Koch MA (2009) Non-coding nuclear DNA markers in phylogenetic reconstruction. *Plant Systematics and Evolution* 282: 257–280.
- Cardini F & Morassi LB (2005) Carotenoid composition and its chemotaxonomic significance in leaves of ten species of the genus *Ceratozamia* (Cycads). *Journal of Plant Physiology* 162(5): 517–528.
- CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences USA* 106: 12794–12797.
- Chase MW, Salamin N, Wilkinson M & Dunwell JM (2005) Land plants and DNA barcodes: short-term and long-term goals. *Philosophical transactions of the Royal Society of London. Series B Biological Science* 360: 1889–1895.
- Dicko HM, Hilhost R, Gruppen N, Alfred ST, Colia L, Willem JHVB & Voragen AGJ (2002) Comparison of content in phenolic compounds, polyphenol oxidase and peroxidase in grains of fifty sorghum varieties from Burkina Faso. *Journal of Agricultural and Food Chemistry* 50: 3780–3788.
- Dische Z & Schwartz D (1973) Thin layer chromatography. *Microchem. Acta*, 2, 13. Cited by: Stahal, E. (ED), 2<sup>nd</sup> edn, Springer Verlage, Berlin.
- Einbond LS, Reynertson K, Luo XD, Basile M & Kennelly E (2004) Anthocyanin antioxidants from edible fruits. *Food Chemistry* 84: 23–28.
- Erlânio OS, Francisco SSB, Fabíola FGR, Adriana RC & José GMC (2012) Chemical composition of the essential oils of *Lantana camara* L. and *Lantana montevidensis* Briq. and their synergistic antibiotic effects on aminoglycosides. *Journal of Essential Oil Research* 24: 447–452.
- Feliner GN & Rosselló JA (2007) Better the devil you know? Guidelines for insightful utilization of nrDNA ITS inspecies-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* 44: 911–991.
- Ford CS, Ayres KL, Toomey N, Haider N, Stahl JV, Kelly LJ, Wikstrom N, Hollingsworth PM, Duff RJ, Hoot SB, Cowan RS, Chase MW & Wilkinson MJ (2009) Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* 159: 1–11.
- Goto T (1987) Structure, stability and color variation of natural anthocyanins. In: Herz W, Grisebach H, Kirby GE & Tamm C (eds) *Progress in Chemistry of Organic Natural Products*. Springer-Verlag, Wien, pp. 113–158.
- Hammer RL (2014) The lantana mess, a critical look at the Genus in Florida. *The Palmetto* 23: 21–24.
- Harborne JB (1967) *Comparative biochemistry of the flavonoids*. Academic Press, London. United Kingdom.
- Hodges DM & Nozzolillo C (1996) Anthocyanin and anthocyanoplast content of Cruciferous seedlings subjected to mineral nutrient deficiencies. *Journal of Plant Physiology* 147: 749–754.
- Kress WJ & Erickson DL (2007) A two-locus global DNA for land plants The coding rbcL gene complements the non – coding trnh-psbA spacer region. *PLoS ONE* 2. e508.
- Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA & Janzen DH (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences USA* 102: 8369–8374.
- Lahaye R, Van Der Bank M, Bogarin D, Warner J, Pupulin F, Gigot G, Maurin O, Duthoit S, Barraclough TG & Savolainen V (2008) DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences USA* 105: 2923–2928.
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* 148: 350–382.
- Liston A, Robinson WA, Oliphant JM & Alvarez-Buylla ER (1996) Length variation in the nuclear ribosomal DNA internal transcribed region of non-flowering seed plants. *Systematic Botany* 21(2): 109–120.
- Lowry OH, Rosebrough NS, Farrand AL & Randall RJ (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* 193: 263–275.
- Marmur J (1961) A procedure for isolation of deoxyribonucleic acid from microorganisms. *Journal of Molecular Biology* 3(2): 208–218.
- Mary LD & Michael TC (2000) Molecular evolution of chalcone synthase multigene family in the morning glory genome. *Plant Molecular Biology* 42: 79–92.
- Metzener H, Rau H & Senger H (1965) Untersuchungen zur synchronisier barkeit einzelner pigmentan angle mutanten von Chlorella. *Planta* 65: 186.
- Niemenak N, Rohsius C, Elwers S, Omokolo ND & Lieberei R (2006) Comparative study of different cocoa (*Theobroma cacao* L.) clones in terms of their phenolics and anthocyanins contents. *Journal of Food Composition and Analysis* 19: 610–619.

- Nørbæk R, Brandt K, Nielsen JK, Ørgaard M & Jacobsen N (2002) Flower pigment composition of *Crocuss* species and cultivars used for a chemotaxonomic. *Biochemical Systematics and Ecology* 30(8): 763–791.
- Ohmiya A (2011) Review diversity of carotenoid in flower petals. *Japan Agricultural Research Quarterly* 45 (2): 163–171.
- Pennisi E (2007) Taxonomy. Wanted: a barcode for plants. *Science* 318: 190–191.
- Poczai P & Hyvnen J (2010) Nuclear ribosomal spacer regions in plant phylogenetics problems and prospects. *Molecular Biology Reports* 37: 1897–1912.
- Porter CH & Collins FH (1991) Species– diagnostic differences in the ribosomal DNA internal transcribed spacer from the sibling species *Anopheles freeborni* and *Anopheles hermsi* Diptera. Culicidae. *The American Journal of Tropical Medicine and Hygiene* 45: 271–279.
- Ragupathy S, Newmaster SG, Balasubramaniam V & Murugesan M (2009) DNA barcoding discriminates a new cryptic grass species revealed in an Ethnobotany study by the hill tribes of the Western Ghats in southern India. *Molecular Ecology Resources* 9: 164–171.
- Savitree M, Isara P, Nittaya SL & Worapan S (2004) Radical Scavenging Activity and Total Phenolic Content of Medicinal Plants Used in Primary Health Care. *Journal of Pharmaceutical Sciences* 9(1): 32–35.
- Sharma OP (1981) A review of the toxicity of *Lantana camara* (Linn) in animals. *Clinical Toxicology* 18 (9): 1077–1094.
- Simona IV, Cornelia P, Laura R & Laslo V (2008) *Separation of pigments from Petunia's petals using thin layer chromatography*. Analele Universităţii din Oradea, Fascicula: *Protecţia Mediului*. Vol. XIII, pp.22-233.
- Soliman M & Amer WM (2002) Atlas: Trees & Flowers Maadi district. Dar El Kutub, Egypt, pp. 40.
- Soltis DE, Mavrodiev EV, Doyle JJ, Rauscher J & Soltis PS (2008) ITS and ETS sequence data and phylogeny reconstruction in allopolyploids and hybrids. *Systematic Botany* 33: 7–20.
- Stanković MS (2011) Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science* 33: 63–72.
- Stintzing F, Stintzing A, Carle R, Frei B & Wrolstad RE (2002) Color and antioxidant properties of cyaniding-based anthocyanin pigments. *Journal of Agricultural and Food Chemistry* 50: 6172–6181.
- Tamura K, Stecher G, Peterson D, Filipski A & Kumar S (2013) MEGA6. molecular evolutionary genetics analysis. *Molecular Biology and Evolution* 30: 2725–2729.
- Tsakadze DM, Samsoniya SA, Ziaev R & Abdusamatov A (2005) Alkaloid and phenolic compounds of *Galanthus caucasicus*, *Magnolia obovata*, *Cocculus laurifolius* and *Veratrum lobelianum* grown in Georgia. *Molecular Diversity* 9: 41–44.
- Wheeler WC & Honeycutt RL (1988) Paired sequence difference in ribosomal RNAs. Evolutionary and phylogenetic implications. *Molecular Biology and Evolution* 5: 90–96.
- White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis MA, Gelfand DH, Sninsky JJ & White TJ (eds) *PCR protocols: a guide to methods and application*. San Diego: Academic Press, pp. 315–322.
- Zhishen J, Mengcheng T & Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64: 555–559.