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Phytochemical screening and *In Vitro* antioxidant activity of *Lantana Camara* L. and *Solanum Elaeagnifolium* C

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Abstract
Medicinal plants being the effective source of both traditional and modern medicines, are genuinely useful for primary health care. *Lantana camara* and *Solanum elaeagnifolium* were pharmacologically evaluated. Phytochemical screening of ethanol and petroleum ether extracts clearly showed the presence of certain important secondary metabolites. Among the two extracts, the ethanol extract was found to be more potent than petroleum ether extract. The antioxidant activity of the plant extracts were determined by using Phospho – molybdenum assay, Nitric oxide free radical scavenging assay and FRAP assay. The results of the present study clearly indicated that Ethanol extract showed better Nitric oxide free radical scavenging activity in *S. elaeagnifolium* at concentration of 1000 mg/ml of extract. Among the two plant material, the *S. elaeagnifolium* was found to be more potential than the *L. camara*.

Keywords: Phytochemical screening, Antioxidant activity, *Lantana camara, Solanum elaeagnifolium*

1. Introduction
Medicinal plants are of noble worthiness to mankind. They are nature's offering for human beings to regulate a sickness free healthful life. They perform a necessary role in preserving our health [3]. Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind [10].
Approximately 80% of the world population depends exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual but in the recent years, more and more people are complementing their treatment with natural supplements [5]. Furthermore, motivation of people towards herbs are increasing due to their concern about the side effect of drugs, those are prepared from synthetic materials. Plants are the good sources for the discovery of pharmaceutical compounds and medicines. Natural products could be potential drugs for humans or livestock species and also these products and their analogues can act as intermediates for synthesis of useful drugs [15]. Plants possess many phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer [21]. Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic [4, 11, 16]. In the present study, to evaluate the phytochemical constituents and *in vitro* Antioxidant activity of *L. camara* and *S. elaeagnifolium* were determined.

2. Materials and Methods
Collection of the sample
Fresh plants leaves of *Lantana camara* L. and *Solanum elaeagnifoilum* C. were collected from the natural strands of Coimbatore district, Tamil Nadu. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of the fruit extracts
Fifty grams of air- dried powder were taken in 200 ml of each different solvents (Petroleum ether- Ethyl acetate and Ethanol/water) in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. Then the supernatants were collected and the solvent was evaporated to make the final volume one fourth of the original volume and stored at 4°C in airtight bottles.

Preliminary phytochemical analysis of various extracts
Qualitative phytochemical screening of Ethanol and Petroleum ether extracts of *Lantana camara* and *Solanum elaeagnifolium* were carried out by using the following standard methods.

Detection of Alkaloids
Mayer's test [7]
Few ml of filtrate, a drop or two ml of Mayer's reagent solution are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Detection of Tannins
Ferric chloride test [14]
The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of tannins.

Detection of Flavonoids
Alkaline reagent test
An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Detection of Phyto sterols and Triterpenoids [8]
Libermann- Burchard's test
The extract (50 mg) is dissolved in 2 ml acetic anhydride. To

this, one or two drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of color changes shows the presence of phytosterols.

Detection of Saponins [13]
The extract (50 mg) is diluted with water and made up to 20 mL. The suspension is shaken in a graduated cylinder for 15 minutes. A 2 cm layer of foam indicates the presence of saponins.

Detection of Glycosides
Fifty mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hr on a water bath, filtered and the hydrolysate is subjected to the following test.

Borntrager's test [7]
Two ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink color indicates the presence of glycosides.

Detection of Fixed oils and Fats [13]
Spot test
A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Detection of Gum and Mucilage [20]
The extract (100 mg) is dissolved in 10 ml of distilled water and to this 25 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

Antioxidant assay
The antioxidant activity of crude extracts was determined by the following *in-vitro* methods.

Phospho-molybdenum assay
The total antioxidant capacity of the extracts were evaluated by the phosphomolybdenum method described by [17]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract, subsequent formation of green phosphate/Mo (V) complex at acidic pH 0.3 ml each extract (6%) in triplicates were combined with 3 ml of reagent solution (0.6 M Sulfuric acid, 28 mm Sodium phosphate and 4mM Ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer (Jenway 6025) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of Ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) with methanol.

Nitric oxide free radical scavenging
Nitric oxide free radical scavenging was carried out as follows. Sodium nitroprusside (10mM) in phosphate buffer (pH 7.7) was incubated with 900 µg/ ml of each extract (6%) dissolved in their respective solvents in triplicate, and the tubes were incubated at 25°C for 120 min. After incubation, 0.5 ml of the reaction mixture was diluted with 0.5 ml of Griess reagent (2% ortho phosphoric acid, 1% sulphanilamide, 0.1% napthylethyl enediamine). Ascorbic acid was used as the standard. The absorbance of the pink chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with N-napthyl ethylenediamine was measured at 546 nm against the corresponding blank solution. The degree of free radical scavenging in the extract was measured by determining the difference in absorbance between the control and the sample and expressed as percentage free radical scavenging of the Nitric Oxide by the extract [9].

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD- Sample OD}}{\text{Control OD}} \text{ X } 100$$

Ferric Reducing Antioxidant Power assay
3.6 ml of FRAP solution (0.3 M of Acetate buffer –pH 3.6; 10 m M of TPTZ in 40 mM of HCl and 10 mM of $FeCl_3.6H_2O$) is added to distilled water (0.4 ml) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 ml) which was taken in triplicates and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of $FeSO_4$ $6H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 m M) were used and the absorbance values were measured as for sample solutions [2].

3. Result and Discussion

Phytochemical screening
Ethanol and Petroleum ether extracts of leaf samples were subjected for qualitative analysis ophytochemical compounds. Ethanolic extract of *L. camara* leaf has positively answered for alkaloids, flavonoids, tannins, steroids, triterpenoids and glycosides while presence of alkaloids, tannins, steroids, triterpenoids and saponins were detected in the ethanolic extract of *S. elaeagnifolium* leaves (Table-1). The medicinal values of these economically important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids and saponin etc [6, 12]. To promote the proper use of phytomedicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way [19].

Table 1: Preliminary phytochemical screening of Ethanol and Petroleum ether extracts of *L. camara* and *S. elaeagnifolium.*

Phytoconstituents	*L. camara* leaf extract		*S. elaeagnifolium* leaf extract	
	Ethanol	Petroleum ether	Ethanol	Petroleum ether
Alkaloids	+	--	+	--
Flavanoids	+	--	--	--
Tannins	+	--	+	--
Steroids	+	-	+	+
Triterpenoids	+	--	+	--
Saponins	--	--	+	--
Glycosides	+	--	--	--
Gum and	--	-	--	--
Mucilage	--	-	--	--
Fixed oils				

(+) Indicates Positive (--) Indicates Negative

Antioxidant activity

Phosphomolybdenum assay

The extracts of all the tested plant specimens possessed total antioxidant activity, but to varying degrees, ranging from 1.5 to 19.4 mg / ml (Table-2). Using the organic solvent extraction, generally Ethanol showed better TAC. A maximum total antioxidant activity was offered by Ethanol extract of *S. elaeagnifolium* (19.4 mg / ml), followed by ethanol extract of *L. camara* (18 mg /mL), Petroleum ether extracts of *L. camara* (3.4 mg / ml) and Petroleum ether extract of *S. elaeagnifolium* (1.5mg / mL) at 1000 mg/ml. which is agreement with that of the previous reports on *Stachys iavandulifolia, Ocimum basilicas* [1]. And *Caesalpinia bonducella* [18].

Table 2: Phospho- Molybdenum Assay of ethanol and petroleum ether extracts of L. *camara* and *S. elaeagnifolium**

Plant species	Total antioxidant activity (mg Ascorbic acid equivalent / ml sample)	
	Plan extract	
	Ethanol	Petroleum ether
L. camara	18.0 ± 0.9	3.4 ± 1.3
S. elaeagnifolium	19.4 ± 1.0	1.5 ± 0.4

* Data are mean of three replicates, ± Standard Error.

Oxide free radical scavenging assay

The nitric oxide free radical scavenging assay of two different plant samples were given in table 3 which was found to be ranged from 0.311 to 3.526%. Among the two different species, the ethanol extracts of *S. elaeagnifolium* (3.526%) have exhibited the highest rate of free radical scavenging activity at 100mg/ml. When compared with standard ascorbic acid, both the samples of the present study exhibited higher reducing power in both the crude extracts at 1000 mg/ml concentration. Which is agreement with that of the previous reports on *Caesalpinia bonducella* [18].

Table 3: Nitric oxide free radical scavenging assay in ethanol and petroleum ether extracts of *L. camara* and *S. elaeagnifolium**

Plant species	Radical scavenging activity (%)	
	Plant extract	
	Ethanol	Petroleum ether
L. camara	0.809 ± 0.18	0.311 ± 0.07
S. elaeagnifolium	3.536 ± 0.76	1.276 ± 0.96

*Data are mean of three replicates, ± Standard Error.

FRAP assay

The total antioxidant activity of the studied plant samples are shown in the table 4.The extracts showed considerable antioxidant activity from 3.5 mg of $FeSO_4$/g of dry plant equivalents in *S. elaeagnifolium* (Petroleum ether extract) to 11.48 mg/g.

Table 4: FRAP Assay ethanol and petroleum ether extracts of *L. camara* and *S. elaeagnifolium**

Plant species	Total antioxidant activity (mg $FeSO_4$ equivalent / g sample)	
	Plant extract	
	Ethanol	Petroleum ether
L. camara	7.65± 0.28	4.08 ± 0.19
S.elaeagnifolium	11.48 ± 0.38	3.5 ± 0.52

*Data are mean of three replicates, ± Standard Error.

4. Conclusion

The results of the present study, clearly explained that among the two plant material, the *S. elaeagnifolium*was found to be more potential than the *L. camara*. Thus, after conducting certain advanced studies, the phytochemical compounds may be separated and biosynthesis and structural elucidations can be carried out in future for the effective utilization of these potential medicinal plants.

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