



## Phytochemical Analysis and *In-vitro* Antioxidant and cytotoxic activity of Aerial parts of *Cestrum aurantiacum* and *Solanum mauritianum* (Solanaceae weeds of Nilgiris)

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### Abstract

To investigate the microscopical, Phytochemical (Qualitative and Quantitative) and *In-vitro* antioxidant studies of hydro alcohol (70%), Aqueous, Ethyl acetate, Chloroform, Pet - ether and total saponin fractions of aerial parts of *Cestrum aurantiacum* (C.aurantiacum) and *Solanum mauritianum* (S. mauritianum). The Phytochemical quantification of the total phenolic, flavanoid and alkaloid contents were estimated using gallic acid, rutin and atropine as standard; Total saponin content was isolated and estimated by using gravimetric analysis; *In-vitro* antioxidant activity was evaluated for extracts by using different free radicals (ABTS and DPPH) assay techniques. S. mauritianum aerial parts hydro- ethanol (70%) extract have more phenolic content, C.aurantiacum aerial parts hydro-ethanol extract have more of alkaloid content, flavanoid and saponin content. The total saponin fractions showed potent IC<sub>50</sub> values of 55.69 ug/ml and 54.99 ug/ml for ABTS assay and IC<sub>50</sub> values of 119.95 ug/ml and 101.68 ug/ml for DPPH assay. The cytotoxic assay was carried for the total saponins on MCF-7 and HCT-116 cell lines and showed potent cytotoxic activity of 212±0.23 ug/mL and 67±1.32 ug/mL on MCF-7 cell lines and on HCT-116 cell lines with 208±0.34 ug /mL and 308±0.47ug/mL. Conclusion: In present study, we found that the selected plants possess good amount of phenolics, alkaloids, flavanoids and saponins, among all the fractions the total saponin fractions showed better antioxidant activity in compare to other fractions, The fractions also showed a good *In-vitro* cytotoxic activity on MCF-7 cell lines and moderate against HCT-116 cell lines in comparison to standard Salvicine

### Keywords

Antioxidant studies, *In-vitro* Cytotoxic assay, *Cestrum aurantiacum*, phytochemical screening and *Solanum mauritianum*

### Introduction

The universal role of plants in the treatment of disease is exemplified by their employment in all the

major systems of medicine irrespective of the underlying philosophical premise. Undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal value, which have yet to be discovered; large numbers of plants are constantly being screened for their possible pharmacological value (particularly for their Antioxidant, Anti-inflammatory, Hypotensive, Hypoglycemic, Amoebicidal, Anti-fertility, Cytotoxic, Antibiotic and Anti-parkinsonism properties). Phytopharmacists with a multidisciplinary background are able to make valuable contributions to these rapidly developing fields of study [1]. The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. These traditional knowledge systems have started to disappear with the passage of time due to scarcity of written documents and relatively low income in these traditions [2]. People return to herbal medicines which have fewer side effects. The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges therefore they are more prone to extinction. Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socio-economic changes [3, 4, 5]. The phytochemical, pharmacological and medicinal value of weed flora of Nilgiris has not been studied much in much depth so far. Hence, the present investigation provides an opportunity to profile chemical and pharmacological properties of the weeds [6].

*Cestrum aurantiacum* (orange Cestrum, "orange Jessamine", orange-flowering Jessamine, and yellow Cestrum) is an extensive ever green solanaceous bushy weed of the Nilgiris. However very less studies was carried out like antibacterial activity of

this plant <sup>[7]</sup>. But so far no phytochemical studies were carried out on this plant. Hence the present studies showed an interesting fact that this plant is a richest source of alkaloids, flavanoids, saponins and phenolics and found to be a source of antioxidant activity. *Solanum mauritianum* also called as *Solanum auriculatum* (Woolly Nightshade, Ear-leaved Nightshade (or "earleaf nightshade"), Flannel Weed, Bugweed, Tobacco Weed, Tobacco Bush, Wild Tobacco and Kerosene Plant) is an extensive ever green solanaceous shrubby weed of the Nilgiris distributed on the fallow lands. The plant is reported for its glycoalkaloidal fractions and having traditional claims for its anticancer activity <sup>[8-9]</sup>. The main objective of our research was to investigate the phytochemical constituents and to find out the potent active phytochemical fractions from these weed plants for antioxidant and anticancer activity.

### Materials and Methods:

#### Chemicals and drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, and Mumbai.

#### Microscopical Authentication

The plant material used in present study was collected from Udthagamandalam in June 2013, The Nilgiris district, Tamilnadu, India and was authenticated by the field taxonomist Dr. S. Rajan, Survey of Medicinal Plants and collection unit, Department of Ayush, Emarlad.

#### Preparation of extracts from aerial parts of *C.aurantiacum* and *S. mauritianum*

The Freshly collected plant material was dried under shade and the dried material was milled to obtain a fine powder. One kg of the powdered weed material was subjected to triple cold maceration using 1500 ml of 70%v/v hydro ethanol as a solvent and the filtrates was distilled and concentrated under vacuum using Rotary evaporator (Buchi R 210). The concentrated extracts were dried in vacuum dessicator and the extractive yield was calculated on dried basis <sup>(10-11)</sup>. 5 gms of hydro-alcoholic (70%v/v) mother extract was dissolved in 25 ml of distilled water and subjected to liquid – liquid fractionation using 50 ml of pet-ether, 100 ml of chloroform, 100 ml of ethyl acetate. The obtained fractions were concentrated under vacuum using Rotary evaporator (Buchi R 120).

#### Qualitative phytochemical Analysis

Preliminary Phytochemical screening of secondary metabolites was carried out for extracts and the

fractions by the method described by Gibbs, 1974 & Harborne, J.B.1973 <sup>[12-13]</sup>.

#### Quantification of Total Phenolic content

The total phenol content of the 70% hydro-alcoholic extract was determined with Folin- Ciocaletau assay using standard solution of gallic acid (10, 20, 40, 60, 80 and 100 mg/l) (Sultana *et al.*,2012)<sup>(14)</sup>.

#### Quantification of the total flavonoid content:

The total flavonoid content was measured with an aluminum chloride colorimetric assay using a solution of standard quercetin (10, 20, 40, 80 and 100 mg/l) (Sultana *et al.*,2012).<sup>(14)</sup>

#### Quantification of the total Alkaloid content:

The total alkaloid content was measured with bromo cresol green colorimetric assay using BCG using a standard solution of atropine (10, 20, 40, 60, 80 and 100 mcg/ml) (Ganapathy *et al.*,2015) <sup>[15]</sup>.

#### Quantification of the total saponin content by gravimetric technique:

5 g of *sample* was used for the separation of total saponins by using gravimetric analysis followed by the method Kareru *et al.*,2008 <sup>[17]</sup>. The separated total saponin fractions were confirmed by phytochemical test and by performing TLC Analysis using n-butanol: Acetic acid: water (40:10:50) and also identified by using FT-IR functional group analysis <sup>[16-18]</sup>.

#### *In-vitro* anti oxidant activity

For the assessment of free radicals scavenging activity, the pet ether, chloroform, ethyl acetate, Aqueous, hydro alcohol (70%v/v) and total saponin fractions were dissolved in water and 5% dimethyl sulphoxide (DMSO) respectively.

#### DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Narender Prasad *et al*<sup>[19]</sup>.

#### ABTS free radical scavenging activity

The ABTS free radical scavenging of the fractions was determined by using Sigma antioxidant assay kit (Catalog no: CS0790) and method followed was described by Chang *et al* <sup>[20]</sup>.

#### *In-vitro* Cytotoxic assay On Mcf-7 Cell Lines and Hct-116 Cell Lines

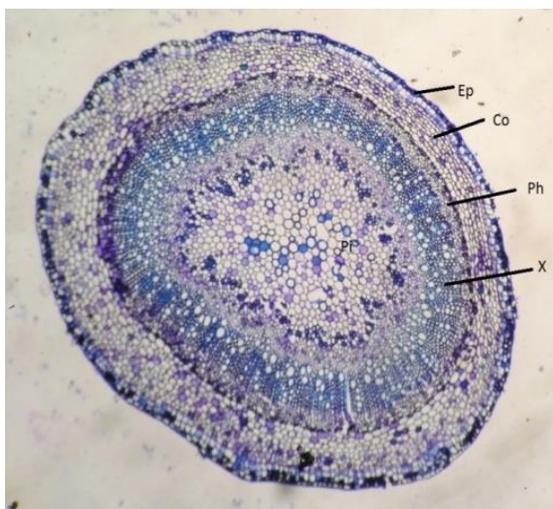
The *In-vitro* cytotoxic assay was carried out using SRB assay followed by the method described by Vijayan *et al.*,2003.<sup>(21)</sup>

## Results and Discussion

### Microscopic Studies

**Stem Microscopy:** The transverse section of the stem showed rough circular outline measuring 1.7 mm in thickness. The stem showed presence of epidermis, cortex, vascular cylinder and wide pith. The epidermal layer is thin and the cells are small elliptical in shape and thick walled. The sub epidermal layer of cells are radially stretched into vertically oblong. These cells divided by periclinal walls producing the peridermis. The cortical zone is 100  $\mu$ m thick, it is parenchymatous and the cells are circular and thin walled.

**Fig.1.** Transverse Section of Stem of *Cestrum aurantiacum*. Co – Cortex, ep – Epidermis, IPH – Inner phloem, oph – outer phloem, pi – pith, sc – sclerenchyma, sep – Sub epidermis, x – xylem.

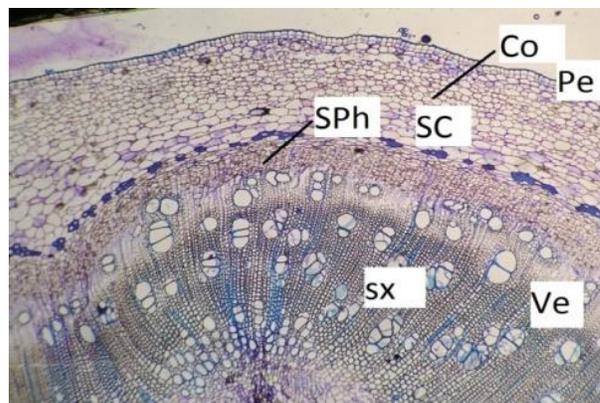


**Transverse sections of aerial parts of *Solanum mauritianum***

### Stem Microscopy

The transverse section of the stem showed a circular and even in sectional view and it is 3.6 mm thick and it consists of epidermal layer of radially elongated cells with thick cuticle. The epidermal cells have undergone tangential division forming an initial periderm cells towards inside. Fig.2

**Fig.2.** Transverse Section of Stem of *Solanum mauritianum*. Co – cortex, pe – periderm, phr – phloem ray, Sc – sclerenchyma, Sph – secondary phloem, sx – secondary xylem, ve- vessels, XF – xylem fibre, XR- xylem Ray



### Qualitative Phytochemical Analysis

Both the plants showed the presence of important metabolites like Alkaloids, Terpenoids, Glycosides, Saponins, Tanins and Phenolics.

### Quantitative Analysis

*S. mauritianum* found to contain  $119.94 \pm 0.63$  mcg/g gallic acid equivalent,  $59.44 \pm 0.13$  mcg/g quercetin equivalent,  $163.10 \pm 0.41$  mcg/g atropine equivalent and 1.42% w/w of saponin content. *C. aurantiacum* found to contain  $99.33 \pm 0.14$  mcg/g gallic acid equivalent,  $149.19 \pm 0.25$  mcg/g quercetin equivalent,  $188.16 \pm 0.22$  mcg/g atropine equivalent and 13.4 % w/w saponin content. The data is represented in the Table1

### Antioxidant Assay

The total Saponin fractions of *C. aurantiacum* and *S. mauritianum* showed potent and highest  $IC_{50}$  values in compare to other extracts and fractions, found to be 55.42  $\mu$ g and 119.95  $\mu$ g (ABTS and DPPH) for *C. aurantiacum* total saponin fraction and 51.23  $\mu$ g and 101.68  $\mu$ g ( ABTS and DPPH) for *S. aurantiacum* total saponin fraction. The data is represented in the Table2.

### In-vitro cytotoxic assay on MCF-7 and HCT-116 cell lines

The cytotoxic assay was carried for the total saponins on MCF-7 and HCT-116 cell lines and showed potent cytotoxic activity of  $212 \pm 0.23$   $\mu$ g/mL and  $67 \pm 1.32$   $\mu$ g/mL on MCF-7 cell lines and on HCT-116 cell lines with  $208 \pm 0.34$   $\mu$ g /mL and  $308 \pm 0.47$   $\mu$ g/mL. The data was represented in the Table 3

### Discussion

The qualitative analysis revealed that the aerial part extracts of *C. aurantiacum* and *S. mauritianum* consists of carbohydrates, alkaloids, steroids, phenols, glycosides etc. These secondary metabolites from plants are known to be useful in the treatment of many diseases. Thus, these plants which contain

important metabolites may serve as a potential source for the treatment of diseases. These plants are considered as an extreme weeds in the Niligiris and not explored phytochemically and pharmacologically, this present research work proved that these plants contains important secondary metabolites like alkaloids, glycosides, flavanoids, phenolics and saponins. The quantitative analysis of these extracts proved that these plants are potent sources for

phenolics, alkaloids and flavanoids which are potent antioxidant sources where there is a major research is going on to find out for novel compounds as a sources for antioxidants and the saponin fractions showed a good anticancer activity on MCF-7 breast cancer cells, further these compounds have to study in extensive to establish the chemical structures and molecular mechanism. Further these weeds can be good sources as future anticancer drug discovery.

**Table 1 Quantitative Analysis of selected weeds**

S.NO	Name of the weed	Total Phenol content (Mcg/g Gallic acid equivalent DW)	Total Flavanoid content (Mcg/g Quercitin equivalent DW)	Total Alkaloid content (Mcg/g Atropine equivalent DW)	Total Saponin content (%w/w) based on gravimetry
1.	<i>Cestrum aurantiacum</i>	99.33±0.4	149.19±0.2	188.16±0.93	13.4±0.17
2.	<i>Solanum mauritianum</i>	119.94±0.31	59.44±0.42	163.10±0.23	1.42±0.16

**Table 2 ABTS AND DPPH assay of *Cestrum aurantiacum* aerial part extract and various fractions**

S.No	Name of the fraction	ABTS ASSAY IC50 VALUES (ug/ml)	DPPH ASSAY IC50 VALUES (ug/ml)
1.	<i>Cestrum aurantiacum</i>	80.87	764.04
a.	Pet ether		
b.	Chloroform	57.13	156.29
c.	Ethyl acetate	59.90	145.37
d.	Aqueous	58.73	87.35
e.	Total Saponin	55.19	119.95
f.	Hydro alcohol extract	55.69	85.0
g.	STD Rutin	5.66	14.16

**Table 3 ABTS AND DPPH assay of *Solanum mauritianum* aerial part extract and various fractions**

S.No	Name of the fraction	ABTS ASSAY IC50 VALUES (ug/ml)	DPPH ASSAY IC50 VALUES (ug/ml)
1.	<i>Solanum mauritianum</i>	56.87	75.28
a.	Pet ether		
b.	Chloroform	62.58	533.04
c.	Ethyl acetate	66.70	143.67
d.	Aqueous	56.78	140.11
e.	Total Saponin	54.99	101.68
f.	Hydro alcohol extract	55.59	109.29
g.	STD Rutin	5.66	14.16

**Table 4 Determination of cytotoxicity by SRB method**

Compound	CTC <sub>50</sub> (µM) on MCF-7	CTC <sub>50</sub> (µM) on HCT-116
C. aurantiacum	212±0.23 ug/mL	208±0.34
S. mauritianum	67±1.32 ug/mL	308 ±0.47
Salvicine	2.61±1.05	117 ± 1.34

CTC<sub>50</sub>-concentration of the sample required to kill 50% of the cell Mean ± SD

**Conflict of interest statement**

We declare that we have no conflict of interest.

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