ANTI TUMOR ACTIVITY AND IN-VIVO ANTIOXIDANT STATUS OF HYPTIS SUAVEOLENS AGAINST EHRLICH ASCITES CARCINOMA IN SWISS ALBINO MICE

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ABSTRACT

In the present work anti tumor and antioxidant potential of stem and leaves of HypHs suaveolens against Ehrlich Ascites carcinoma (EAC) was studied employing In-Vivo methods. To determine the antitumor potential parameters studied are Tumor volume, Packed cell volume, viable and non-viable cell count and life span of the host. The ethanolic extract of Hyptis suaveolens (EEHS) was administered at a dose of 100,200,300,mg/kg bw. once a day for 14 days after 24hrs of tumor induction. The treatment with EEHS increased the mean survival time. Haematological studies of tumor bearing mice revealed the decreased levels of Hemoglobin, RBC count and increased WBC count, administration of plant drug treatment reverted back to normal. In the antioxidant studies the EEHS decreased the LPO level and increased the level of glutathione, SOD and CAT. The result suggested that the EEHS played significant role in reducing tumor by increasing the life span and by cleaving the free radicals.

Keywords: Ethanolic extract of Hyptis suaveolens (EEHS), Ehrlich Ascites carcinoma (EAC), Malondialdehyde (MDA), Glutathione, SOD, Catalase, LPO.

INTRODUCTION

Cancer is the second killer disease, to combat this serious disease doctors and scientists are focusing their researches towards discovering a drug from alternate medicines, as there exist no definite cure in modern medicine, serious attempts were made towards discovering a molecule from herbal source which resulted in the isolation and characterization of anti cancerous chemotherapeutic agents such as vincristine, vinblastine (alkaloid), Podophylootoxin (lignins), and taxol (diterpenoid). With a view to develop a novel terpenoid anti cancerous drug from plant sources a botanical survey of anti cancerous plants in and around Trichy was conducted which resulted in the selection of a rich diterponid Lamiaceae member Hyptis suaveolens.

Hyptis suaveolens (Lamiaceae) is an erect aromatic herb and commonly known as Kanathulasi/Ramathulasl. It is an under shrub present in almost all parts of India. Traditionally this plant is used as a stimulant, carminative, sudorific and lactagogue. Leaf juice used for healing wounds and has agreeable remedy in case of cold, coughs, consumption and lung complaints. Its chemistry is also interesting as it yielded terpenoid compounds such as ursoic acid and suaveolic acid etc. There was no experimental proof to reveal the antitumor and antioxidant potential of this plant. Hence in the present study attempts were made to evaluate the anti tumor and antioxidant potential of the selected plant under study.

MATERIALS AND METHODS

Plant Collection and Extraction

The aerial parts of Hyptis suaveolens were collected from in and around Trichy in the month of December 2006 and identified with the help of Flora of Presidency of Madras and authenticated...
with RAPINAT Herbarium, Department of Botany, St. Joseph's college, Trichy. The plant material was shade dried and pulverized. About 500gms of plant material was soaked in the ethanol for 48h. The solvent distilled off under reduced pressure at 50° and dried in vacuum (yield: 4.5% w/w) and dissolved in isotonic normal saline and used for the study.

Animals

Male Swiss albino mice of about 7-8 weeks of age with an average body weight of 25±2g were used for the experiment. The animals were procured from Tamil Nadu Veterinary University Chennai. They were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12h dark/light cycle). They were fed with standard laboratory diet and were given sterilized water *ad libitum*. The animals were maintained in standard Animal house CPCSEA approval no:790/03/ac/CPCSEA. The study was conducted after obtaining the necessary clearance from Institutional Animal Ethical Committee.

Cells

EAC cells were obtained through the courtesy of Amla Cancer Research Centre, Thrissur and were maintained by weekly intraperitoneal inoculation of 1X10^6 cells/mouse.

Effect of EEHS on Short Time *In-Vitro* Cytotoxicity

Short-term cytotoxicity was assessed by incubating 1X10^6 EAC cells in 1 mL phosphate buffer saline with varying concentrations of the EEHS at 37° for 3 h in CO_2_ atmosphere ensured using a McIntosh field jar. The viability of the cells was determined by the trypan blue exclusion method.

Effect of EEHS on survival Time

The Swiss albino mice were divided into five groups of six animals each. Animals were inoculated with 1X10^6 cells/mouse on day 0 and treatment with EEHS started after 24 h of tumor inoculation at a dose of (100,200,300mg/kgbw p.o.) to group 2, 3, 4 respectively for 14 days. Group 5 animals were administered with 5-Fluorouracil (standard drug) 20mg/kgbw for 14 days. Group 1 animals served as tumor control and received normal saline. The percentage increase in life span was calculated as follows.

\[
\text{ILS} \, (\%) = \frac{\text{Mean survival time of treated group}}{\text{Mean survival time of control group}} - 1 \times 100
\]

\[
\text{MST} = \frac{\text{Day of first death} + \text{day of last death}}{2}
\]

Experimental Protocol

The mice were divided into 6 groups of six each.

GROUP I - Normal control

GROUP II - Ehrlich Ascites Carcinoma cell line (1X10^6 cell/mouse)

GROUP III - Ehrlich Ascites Carcinoma cell line (1X10^6 cells) treated with 100mg/kg bw of the ethanolic extract *Hyptis suaveolens*.

GROUP IV - Ehrlich Ascites Carcinoma cell line (1X10^6 cells) treated with 200mg/kg bw of the ethanolic extract *Hyptis suaveolens*.

GROUP V - Ehrlich Ascites Carcinoma cell line (1X10^6 cells) treated with 300mg/kg bw of the ethanolic extract *Hyptis suaveolens*.

GROUP VI - Ehrlich Ascites Carcinoma cell line (1X10^6 cells) treated with 5-Fluorouracil (5-FU) (20mg/kgbw) (Standard Drug)

Treatment started after 24 h of EAC inoculation. The plant extract administered orally for 14 days. After the experimental period animals were sacrificed by cervical decapitation, blood was collected. The liver was washed with ice cold saline and used for the antioxidant studies.

Tumor Volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge
tube and packed cell volume was determined by centrifuging at 1000rpm for 5min.

**Tumor Cell Count**

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares were counted.

**Viable/Non-Viable Tumor Cell Count**

The cells were then stained with trypan blue (0.4% in normal saline) dye.

**Hematological Parameters**

At the end of the experimental period, animals were allowed overnight fasting following day the animals were sacrificed by cervical decapitation. Blood was collected and used for the estimation of Hemoglobin (Hb) content, red blood cell count (RBC) and white blood cell count (WBC). WBC differential count was carried out using Leishman stained blood smears.

**Estimation of in vivo Antioxidants**

After drawing necessary blood by cervical decapitation, liver was dissected out, rinsed in ice-cold normal saline solution, blotted, dried and weighed. The liver tissue was homogenized using cold 0.15 M Tris-HCl (pH 7.4). A 10% w/v homogenate was used for the estimation of lipid peroxidation (LPO) and reduced glutathione (GSH). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD), Catalase (CAT) and total protein.

**Estimation of Lipid Peroxidation (LPO)**

As a marker for lipid peroxidation, the levels of Thiobarbituric acid reactive substances (TBARS) in the liver was measured by the method of Ohkawa et al., A mixture of 0.4 mL of 10% liver homogenate, 1.5 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of 20% acetate buffer (pH 3.5) and 1.5 mL of 0.8% TBA solution was heated at 95°C for 1 h. After cooling, 5.0 mL of n-butanol-pyridine (15:1) was added, and the absorbance of the n-butanol-pyridine layer was measured at 532 nm.

**Estimation of glutathione (GSH)**

The GSH was determined by the method of Beutler and Kelly. 0.2 mL of tissue homogenate was mixed with 1.8 mL of EDTA solution. To this 3.0 mL of precipitating reagent TCA was added mixed thoroughly and kept for 5 min before centrifugation. To 2 mL of filtrate, 4.0 mL of disodium hydrogen phosphate solution and 1.0 mL of DTNB (5,5-dithio bis 2-nitro benzoic acid) reagent were added and the absorbance read at 412 nm.

**Assay of SOD**

The activity of SOD in tissue was assayed by the method of Kakkar. The assay mixture contained 1.2 mL sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1 mL phenazine methosulphate (186 mol/L), 0.3 mL nitro blue tetrazolium (300 mol/L), 0.2 mL NADH (780 mol/L) and diluted enzyme preparation and water in a total volume of 3 mL. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was allowed to stand for 5 min before reading at 560 nm against n-butanol. The chromogen in the butanol layer was measured at 560 nm against n-butanol.

**Assay of Catalase**

Catalase activity was assayed according to the method of Maehly and Chance. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H₂O₂ and the enzyme extract. The specific activity of Catalase is expressed in terms of units/mg of protein. A unit is defined as the velocity constant per second.
Estimation of Total Protein

The protein content of the tissue homogenate measured by Lowry's method\(^3\). 0.5mL of tissue homogenate was mixed with 0.5mL of 10% TCA and centrifuged for 10min. The precipitate obtained was dissolved in 1.0mL of 0.1N NaOH. From this 0.1mL used for the protein estimation.

Statistical Analysis

The experimental results were expressed as mean±S.E.M. Data were assessed by the method of analysis of ANOVA followed by t-test. \(P<0.001\) was considered as statistically significant.

RESULTS AND DISCUSSION

Effect of EEHS on In-Vitro Cytotoxicity

The ethanolic extract of Hyptis suaveolens showed a significant In-Vitro cytotoxicity at the dose level of 125 mcg/mL.

Effect of EEHS on Mean Survival Time

From the Table I it is noted that in the EAC control group mean survival time was 21 days while the survival time increased to 25 (100mg/kg), 29 (200mg/kg), 31 (300mg/kg) days respectively in EEHS treated groups.

Effect of EEHS on Tumor Response

Anti tumor activity of the EEHS was assessed by the parameters such as tumor volume, Packed cell volume and viable & non viable cell counts which is determined by the nature of stained Trypan blue. The results were given in the Table II. The tumor cell volume, Packed cell volume and viable cell counts increased in the tumor bearing animals compared to normal. Administration of EEHS at the dose of 100mg/kg, 200mg/kg and 300mg/kg significantly decreased the tumor volume, packed cell volume and viable count and increased the non viable cell counts.

Effect of EEHS on Haematological Parameters

Haematological parameters (Table III) of tumor bearing mice such as decrease in Hemoglobin content, RBC count and increase in WBC count were brought back to normal and Lymphocytes, Neutrophils were also altered to normal level in the treated animals.

Effect of EEHS on LPO Level

Reactive oxygen species (ROS) formed in the cancer tissue resulted in the lipid peroxidation and subsequently increased the Malondialdehyde (MDA) level. Fig.1 depicts the level of LPO in experimental animals. In the present study the MDA level significantly increased in the EAC control animals. MDA level was reduced after the drug treatment.

Effect of EEHS on Glutathione Level

Fig.2 illustrates the level of glutathione in experimental animals. The level of glutathione was significantly reduced in EAC bearing mice compared to the normal control group. The level of glutathione increased in dose dependent manner on administration of EEHS.

Effect of EEHS on SOD Level

Fig.3 demonstrate the activity of SOD in liver tissue of experimental animals. There was a significant reduction in the level of SOD in EAC bearing mice, whereas on treatment the SOD level was increased.

Effect of EEHS on Catalase Level

Fig.4 showed the activity of Catalase in experimental animals. The significant reduction in the activity of the Catalase was found in the tumor bearing mice. Feeding with various concentration of EEHS improve the activity of the Catalase.

Hyptis suaveolens a traditional drug source is collected from in and around Trichy identified and studies were carried out for determining the anticancer and antioxidant potential of the test drug against Ehrlich ascites carcinoma cell lines.

In EAC mice a regular rapid increase in the ascites tumor volume was observed. Ascites fluid is
Table I: Effect of EEHS Treatment on the Survival Time of the Tumor Bearing Mice

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Mean survival time (MST) (days)</th>
<th>Increase in life span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC control</td>
<td>21 ±0.02</td>
<td>-</td>
</tr>
<tr>
<td>EAC control + EEHS (100mg/kgbw)</td>
<td>25±0.056*</td>
<td>19.5</td>
</tr>
<tr>
<td>EAC control + EEHS (200mg/kgbw)</td>
<td>29±0.2*</td>
<td>38</td>
</tr>
<tr>
<td>EAC control + EEHS (300mg/kgbw)</td>
<td>34±0.13*</td>
<td>61.5</td>
</tr>
<tr>
<td>EAC control + 5FU (20mg/kg bw)</td>
<td>39±0.10*</td>
<td>85.7</td>
</tr>
</tbody>
</table>

Values are mean S.E.M., n=6

*p<0.001 statistically significant when compared to EAC control group

EEHS: Ethanolic Extract of Hyptis suaveolens
EAC: Ehrlich Ascites Carcinoma

Table II: Effect of EEHS Treatment on Tumor Growth

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Tumor volume (mL)</th>
<th>Packed cell volume (mL)</th>
<th>Viable cells</th>
<th>Non-viable cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>3.3±0.20</td>
<td>1.9±0.12</td>
<td>7.5±0.20</td>
<td>0.45±0.01</td>
</tr>
<tr>
<td>Group III</td>
<td>2.7±0.17*</td>
<td>1.4±1.24*</td>
<td>5.1±0.20*</td>
<td>0.83±0.73*</td>
</tr>
<tr>
<td>Group IV</td>
<td>1.8±0.19*</td>
<td>1.0±0.15*</td>
<td>4.2±0.25*</td>
<td>0.91±0.51*</td>
</tr>
<tr>
<td>Group V</td>
<td>1.2±0.32*</td>
<td>0.6±0.37*</td>
<td>3.4±0.17*</td>
<td>0.95±0.65*</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.8±0.11*</td>
<td>0.4±0.14*</td>
<td>2.2±0.11*</td>
<td>0.99±0.57*</td>
</tr>
</tbody>
</table>

Values are mean S.E.M., n=6

*p<0.001 statistically significant when compared to EAC control group
Viable cells: Not Stained with Trypan blue
Non-viable cells: Stained with Trypan blue

Table III: Effect of EEHS on Haematological Parameters

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Hb content (g/dl)</th>
<th>RBC count 10^6 cells/mm³</th>
<th>WBC count 10^6 cells/mm³</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>13.8±0.35</td>
<td>5.7±0.12</td>
<td>5.6±0.40</td>
<td>78±1.4</td>
<td>29±1.2</td>
<td>1.8±1.5</td>
</tr>
<tr>
<td>Group II</td>
<td>11.7±0.22*</td>
<td>4.3±0.70*</td>
<td>9.8±0.21*</td>
<td>29±1.5*</td>
<td>65±1.4*</td>
<td>2.1±0.5*</td>
</tr>
<tr>
<td>Group III</td>
<td>12.1±0.55b</td>
<td>4.4±0.53b</td>
<td>8.6±0.33b</td>
<td>37±1.1b</td>
<td>51±1.8b</td>
<td>1.79±1.2b</td>
</tr>
<tr>
<td>Group IV</td>
<td>12.5±0.67b</td>
<td>4.7±0.42b</td>
<td>7.1±0.08b</td>
<td>53±1.6b</td>
<td>39±1.1b</td>
<td>1.61±1.34b</td>
</tr>
<tr>
<td>Group V</td>
<td>14.1±0.51b</td>
<td>5.1±0.48b</td>
<td>6.5±0.22b</td>
<td>65±1.4b</td>
<td>31±0.9b</td>
<td>1.40±1.5b</td>
</tr>
<tr>
<td>Group VI</td>
<td>13.4±0.37b</td>
<td>4.9±0.39b</td>
<td>5.4±0.06b</td>
<td>69±1.5b</td>
<td>30±0.1b</td>
<td>1.7±0.8b</td>
</tr>
</tbody>
</table>

Values are mean S.E.M., n=6

*a p<0.001 statistically significant when compared with normal group
*b p<0.001 statistically significant when compared with EAC group
Table IV: Effect of EEHS on Enzymatic and Non Enzymatic Antioxidants

<table>
<thead>
<tr>
<th>Particulars</th>
<th>LPO nmol MDA-mg⁻¹ (protein)</th>
<th>GLUTATHIONE mg⁻¹ (wet tissue)</th>
<th>SOD U-mg⁻¹ (protein)</th>
<th>CATALASE U-mg⁻¹ (protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.96±0.02</td>
<td>2.35±0.09</td>
<td>4.49±0.35</td>
<td>26.4±0.07</td>
</tr>
<tr>
<td>Group II</td>
<td>3.16±0.04*</td>
<td>0.91±0.07*</td>
<td>1.56±0.26*</td>
<td>11.4±0.04*</td>
</tr>
<tr>
<td>Group III</td>
<td>2.60±0.01**</td>
<td>1.15±0.06**</td>
<td>2.28±0.31**</td>
<td>14.7±0.06**</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.13±0.02**</td>
<td>1.69±0.09**</td>
<td>2.97±0.28**</td>
<td>17.2±0.08**</td>
</tr>
<tr>
<td>Group V</td>
<td>1.43±0.03**</td>
<td>2.07±0.25**</td>
<td>3.61±0.35**</td>
<td>20.3±0.07**</td>
</tr>
<tr>
<td>Group VI</td>
<td>1.24±0.01**</td>
<td>2.22±0.08**</td>
<td>4.15±0.22**</td>
<td>25.7±0.07**</td>
</tr>
</tbody>
</table>

Values are mean S.E.M. n=6
*p<0.001 statistically significant when compared with normal group
*p<0.01 statistically significant when compared with EAC group

Fig. 1: Anti Tumor Activity of Ethanolic extract of Hyptis suaveolens on MDA Level,
Values are mean ± S.E.M., (n=6)
* Values are statistically significant at p<0.001
** Values are statistically significant at p<0.01

Fig. 2: Antitumor Activity of Ethanolic Extract of Hyptis suaveolens on Glutathione Level
Values are mean ± S.E.M., (n=6),
* Values are statistically significant at p<0.001
** Values are statistically significant at p<0.01

Fig. 3: Anti Tumor Activity of Ethanolic Extract of Hyptis suaveolens on SOD Level,
Values are mean ± S.E.M., (n=6),
* Values are statistically significant at p<0.001
** Values are statistically significant at p<0.01

Fig. 4: Anti tumor Activity of Ethanolic Extract of Hyptis suaveolens on Catalase Level,
Values are mean ± S.E.M., (n=6),
* Values are statistically significant at p<0.001
** Values are statistically significant at p<0.01

- Normal control
- EAC control
- EAC + EEHS (100mg/Kg bw)
- EAC + EEHS (200mg/Kg bw)
- EAC + EEHS (300mg/Kg bw)
- EAC + 5-FU (20mg/Kg bw)
the direct nutritional source for tumor cells and a rapid increase in the ascites fluid directly represent the tumor growth\(^\text{16}\). Treatment with EEHS at various dose level inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. One of the criteria for determining the anticancer potential of the test drug is the prolongation of the life span of the experimental animal\(^\text{17}\).

In cancer chemotherapy the major problem that are being encountered are myelosupression and anemia\(^\text{18,19}\). The anemia was encountered in the tumor bearing mice mainly due to reduction in the RBC and haemoglobin content and this may be due to iron deficiency or haemolytic conditions\(^\text{20}\). Treatment with EEHS brought back the Haemoglobin, RBC & WBC content more or less normal. This clearly indicates that EEHS possess protective role on the haemopoietic system.

Malondialdehyde is formed during oxidative degeneration of membrane as product of free oxygen radicals\(^\text{21}\), which is an indicator for lipid peroxidation. MDA the end product of lipid peroxidation was reported to be higher in cancerous tissue than normal tissue. Present findings indicated that the level of LPO in cancerous tissue was higher and was brought back to normal after the treatment. The plant drug may reduce the free radicals which resulted in the subsequent decrease in the membrane damage and MDA level.

Glutathione a potent inhibitor of neoplastic process plays a key role in the endogenous antioxidant system. The level of glutathione in drug treated animals was found to be higher which facilitate the inhibition of neoplastic process and may prevent the tumor growth.

SOD and Catalase was found in all oxygen metabolizing cells and the main function is to provide the defense against the potentially damaging activities of Super oxide and hydrogen peroxide. A decrease in SOD activity in EAC bearing mice is due to the loss of Mn SOD activity in EAC cells and the loss of mitochondria\(^\text{22}\). The administration of EEHS at different dose increased the activities of SOD and Catalase in drug treated animals. The lowering of lipid peroxidation and increase in levels of GSH, SOD and Catalase in EEHS-treated animals indicated its inhibiting activities of intracellular oxidative stress resulting due to EAC induction.

The data of the results compared with standard drug 5-fluorouracil (20mg/kg bw). It is observed that the does level of 300mg/kg bw was almost similar to that of standard drug.

**CONCLUSION**

The present study proved the antitumour and antioxidant potentials of ethanolic extract of *Hyptis suaveolens*. The drug treatment increased the lifespan and restored the altered levels of hematological parameters and enzymatic and non enzymatic antioxidants in the liver tissue of EAC bearing mice.

**ACKNOWLEDGEMENT**

Department of Biochemistry, the Management and the Principal for providing necessary facilities to carry out the research work.

**REFERENCES**

Quality control studies on "Virali" - a Siddha drug

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ABSTRACT
In the present paper few aspects of quality control studies on a Siddha drug source "Virali" (Dodonaea viscosa (L.) Jacq.) was carried out. In Ayurveda this plant is used as a substitute for Rasna. The parameters determined in the present study are macro and microscopic standards, physicochemical values, TLC profiles, fluorescence features, and inorganic contents. The data revealed the presence of considerable amount of pharmacologically active compounds and inorganic compounds such as calcium, iron, zinc and potassium. Review of literature showed that this drug is well worked from chemical and biological point of view but much work is not recorded on the pharmacognostic or quality parameters of this interesting drug source. Hence in the present work attempts were made to study this plant drug from pharmacognostic point of view.

Key words: Traditional uses of Virali, macro and microscopic standards, physicochemical values, analytical studies, thin layer chromatographic profiles, microbial counts.

INTRODUCTION

Botanicals are widely used as dietary supplements and medicine. 80% of the world's population depends on plants for their health and skin care. But quality assurance studies in the area of these herbal products are relatively limited and unfocused. Efficacy and safety of many widely used botanical components have not been adequately evaluated. The growth of herbal pharma industry in India is not as sound as in China. The main reason attributed for this is poor quality management of herbal drug production from raw material standardization to finished products. Hence in the present work an indigenous drug "Virali" a common wound healer and an unique plant used by Siddhars in alchemy is studied from quality parameters point of view.

"VIRALI" is botanically equated as Dodonaea viscosa (L.) Jacq. belonging to the family Sapindaceae, an indigenous medicinal herb widespread through out the tropical regions. It is common in dry land and scrub jungles. It is a bushy shrub with simple, sub sessile leaves and yellow resin exudates. Flowers greenish - yellow, in cymes, capsule membranous winged.

The plant is mainly used for inflammation, swellings, wound, gout and rheumatism. In Ayurveda the plant is used as substitute for "Rasna" [14]. In Siddha, it finds use in "Alchemy" and as a "Muppu" drug. The plant yielded Saponin mixture that exhibit anti exudative, phagocytosis enhancing and molluscicidal activities [12]. It is rich in flavonoid alizarin and caryophyllene was the major constituent of essential oil [11]. It also contains isorhammentin, kaempferol 3,7-dimethylether and 3,4,7, trimethyl ether and hautriwaic acid [10]. Even though it is cardio inhibitory [3] and coronary constricting [3], it finds use as a spasmytic, sedative, hypotensive, antimalarial and antibacterial agent [3]. A diterpenoid Dodonic acid and a new flavonoid Viscosol were isolated from aerial part of the plant material.

This Siddha drug source "VIRALI" which is interesting both biologically and chemically is not studied from quality control point of view. Hence attempts were made in the present work to determine the botanical and analytical standards.

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which will contribute towards the proper identification and quality check of this raw drug in the market.

MATERIALS AND METHODS

The aerial parts of Dodonaea viscosa (L.) Jacq. were collected from in and around Trichy in the month of January 2007 and identified using the Flora of Presidency of Madras [6] and authenticated with the help of specimen deposited at Rapinant Herbarium Department of Botany, St. Joseph's College, Trichy. The collected materials were cleaned, shade dried and coarsely powdered. Free hand and microtome sections of the fresh leaf, stem and root were taken and double stained and photo micrographs were taken as per standard procedures [9, 13].

The coarsely powdered material was subjected to hot extraction using Soxhlet apparatus employing various organic solvents in the order of increasing polarity. Physicochemical values [1], behavior of drug powder with various chemical reagents [5], preliminary qualitative analysis [7], thin layer chromatographic profile [7] and fluorescence features [4] were studied and the data of the results obtained were presented. Total microbial counts and presence of lead cadmium and arsenic were tested as per WHO guidelines [2]. The inorganic analysis of the powder was carried out employing Atomic Absorption Spectroscopy (Model 1929, UK) [8].

RESULTS AND DISCUSSION

1. Macroscopic characters (Figure 1)
   1. Leaves-dark green narrow linear alternate, top glossy under side lighter.
   2. Fruit-3-Sided pod containing 3-seeds with flat papery wings.
   3. Exposed trunk with gnarled character when pruned up.
   4. Roots Thick

2. Microscopic characters
   LEAF: C.S. of leaf (FIG II) revealed following salient features
   1. Epidermal layer with thick cuticle
   2. Dense palisade zone
   3. Vascular bundles insulated by sclerenchymatous sheath
   4. Midrib of the leaf is characteristic having smooth hemispherical adaxial side and conical more prominent abaxial side.
   5. The vascular tissue occur in abaxial main and adaxial accessory bundles.

STEM

Transverse sections of fairly matured stem wood and bark were taken and studied after making suitable microscopic preparations. Following important microscopic characters (Figure 3 A & B) were observed

Presence of Radially oblonged thin walled Phellem cells and characteristic phloem rays. Vessels are in radial multiples of 2-6, with scanty axial parenchyma. Xylem fibres thick walled.

Root

Transverse section of a portion of mature root wood (Figure 4 A & B) revealed following characteristic features

1. Semi ring porous wood is similar to stem wood in certain aspects.
2. Circular or oval xylem vessels.
3. Primary xylem crushed to the centre.
4. Xylem fibres and vessel elements are distinct.
5. Xylem parenchyma present.

Figure 2. C.S. of leaf Abs- Abaxial side; Ads - Adaxial side; PM- Palisade Mesophyll; SM - Spongy Mesophyll; Ep- Epidermis; La- Lamina; MS- Median strand; MT- Mesophyll tissue; Ph- Phloem; Sc- Sclerenchyma; Vs- Vascular strand; X-Xylem; LV- Lateral Vascular bundle
Figure 3A. Stem bark - A portion enlarged PM - Phellem; Pd-Pheloid; PhR-Pholem Ray; SE- Sieve Elements; Sph- Secondary phloem; Pe-Periderm

Figure 3B. Stem wood - A portion enlarged Pa-Paratracheal parenchyma; Scl-Sclerenchyma; V- Vessel; XF-Xylem fibers; XR- Xylem Ray

Figure 4A. Root wood - Portion enlarged C-Cortex; Pe-Periderm; Px-Primary xylem; Sc-Sclerenchyma; Sph-Secondary phloem; Sx-Secondary xylem; V-Vessel; XF-Xylem fibers; XR-Xylem ray.

Figure 4B. Mature root wood - A portion enlarged V-Vessel; XF-xylem fibers; XP-Xylem Parenchyma; XR-Xylem Ray
In the Powder microscopic analysis (Figure 5) following salient microscopic characters were observed:

1. Tailed vessel and xylem fibres with narrow end is very characteristic.
2. Distinct Fibre tracheids and
3. Long Vessel element with simple Perforation plate are other noteworthy features.

![Figure 5. Powder analysis Fi-fibers; Ftr- Fibre tracheids; Pp- Perforation plate; Ve-Vessel element, Ta- Tailed Vessel end](image)

### Table 1. Physicochemical values

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Value % W/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Ash Content</td>
<td>4.1 ± 1.2</td>
</tr>
<tr>
<td>2.</td>
<td>Water soluble Ash</td>
<td>2.6 ± 0.83</td>
</tr>
<tr>
<td>3.</td>
<td>Acid insoluble Ash</td>
<td>1.21 ± 0.32</td>
</tr>
</tbody>
</table>

Where n=5 (The experiments were repeated for 5 times)

### Successive extractive values

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hexane</td>
<td>3.56 ± 0.97%</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>2.2 ± 0.52%</td>
</tr>
<tr>
<td>3.</td>
<td>Ethyl acetate</td>
<td>2.8 ± 0.47%</td>
</tr>
</tbody>
</table>

Where n=5 (The experiments were repeated for 5 times)

### Extractive values as per IP

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water</td>
<td>6.54 ± 1.08%</td>
</tr>
<tr>
<td>2.</td>
<td>Alcohol</td>
<td>9.97 ± 1.17%</td>
</tr>
</tbody>
</table>

Where n=5 (The experiments were repeated for 5 times)

### Behaviour of the drug powder with various chemical reagents answered positively for the presence of flavones, alkaloids, terpenes, quinones, saponins sugar and proteins.

### Table 2. Preliminary phytochemical screening of various extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test for</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Ethyl Acetate</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saponin</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Protein</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>3.</td>
<td>Tannin</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>4.</td>
<td>Sterol</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenes</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>6.</td>
<td>Sugar</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>7.</td>
<td>Flavones</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>8.</td>
<td>Coumarins</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>9.</td>
<td>Quinone</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>10.</td>
<td>Lignin</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>11.</td>
<td>Alkaloid</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

### Table 3. Fluorescence analysis of drug powder

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>After 24 hrs in day light</th>
<th>After 24 hrs in UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Drug powder</td>
<td>Green</td>
<td>Light Green</td>
</tr>
<tr>
<td>2.</td>
<td>Drug powder + 50% H2SO4</td>
<td>Brown</td>
<td>Dark Green</td>
</tr>
<tr>
<td>3.</td>
<td>Drug Powder +aq.1N NaOH</td>
<td>Reddish brown</td>
<td>Dark Green</td>
</tr>
<tr>
<td>4.</td>
<td>Chloroform</td>
<td>Yellow</td>
<td>Dark green</td>
</tr>
<tr>
<td>5.</td>
<td>Ethyl Acetate</td>
<td>Green</td>
<td>Dark green</td>
</tr>
<tr>
<td>6.</td>
<td>Alcohol</td>
<td>Dark Green</td>
<td>Light Green</td>
</tr>
<tr>
<td>7.</td>
<td>Water extract</td>
<td>Dark Green</td>
<td>Light Green</td>
</tr>
</tbody>
</table>

"Virali" botanically equated as Dodonea Viscosa (L) Jacq. finds use in traditional systems of medicines like Ayurveda and Siddha. In the present work this traditional drug source is studied from analytical specification point of
Mineral contents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Minerals</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Organic Carbon (%)</td>
<td>2.18</td>
</tr>
<tr>
<td>2.</td>
<td>Total Nitrogen (%)</td>
<td>0.87</td>
</tr>
<tr>
<td>3.</td>
<td>Total Phosphorus (%)</td>
<td>0.31</td>
</tr>
<tr>
<td>4.</td>
<td>Total Potassium (%)</td>
<td>2.54</td>
</tr>
<tr>
<td>5.</td>
<td>Total Sodium (%)</td>
<td>0.09</td>
</tr>
<tr>
<td>6.</td>
<td>Total Calcium (%)</td>
<td>2.98</td>
</tr>
<tr>
<td>7.</td>
<td>Total Magnesium (%)</td>
<td>1.57</td>
</tr>
<tr>
<td>8.</td>
<td>Total Sulphur</td>
<td>0.15</td>
</tr>
<tr>
<td>9.</td>
<td>Total Zinc (ppm)</td>
<td>2.48</td>
</tr>
<tr>
<td>10.</td>
<td>Total Iron (ppm)</td>
<td>19.49</td>
</tr>
<tr>
<td>11.</td>
<td>Total Manganese (ppm)</td>
<td>4.19</td>
</tr>
<tr>
<td>12.</td>
<td>Total Boron (ppm)</td>
<td>0.04</td>
</tr>
<tr>
<td>13.</td>
<td>Total Molybdenum (ppm)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 6. TLC profile

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Colour of the spot</th>
<th>Rf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Yellow</td>
<td>0.15</td>
</tr>
<tr>
<td>2.</td>
<td>Green</td>
<td>0.18</td>
</tr>
<tr>
<td>3.</td>
<td>Light blue</td>
<td>0.28</td>
</tr>
<tr>
<td>4.</td>
<td>Brown</td>
<td>0.43</td>
</tr>
<tr>
<td>5.</td>
<td>Purple</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Microscopic standards determined include Macro& Microscopic standards as mentioned below:

1. Total ash : Not more than 5%
2. Acid insoluble ash : Not more than 1.5%
3. Water soluble extractive : Not less than 6 %
4. Alcohol soluble extractive : Not less than 9%
5. pH : 6.3
6. Microbial Load :
   Total Bacterial Count : 1.1 x 10^4 (WHO limits: Not more than 105)
   Total Fungus Count : Nil
   Enterobacteriaceae : Nil
   Salmonella Species : Nil
9. Heavy Metals :
   Lead : Nil
   Mercury : Nil
   Cadmium : Nil
   Arsenic : Nil

Botanical standards determined include Macro& Microscopic standards as mentioned below:

Macroscopic - Narrow linear alternate leaves with glossy uppersurface. Fruit 3 sided pod and seeds with flat papery wings. Roots thick.

Organoleptic - No characteristic odour, Bitter taste, Slightly aromatic
Physicochemical values data of the drug powder (Table 1) revealed higher extractive values for hexane which indicated the presence of volatile compounds. Alcohol solubility is higher compared to water solubility.

Behavior of drug powder with various chemical reagents revealed the presence of alkaloids, flavonoids, terpenes, coumarins, quinones and sterols. Preliminary phytochemical screening of various extracts of the drug powder also (Table 2) confirmed the presence of alkaloids, coumarins, sterols and terpenes.

Fluorescence analysis of the drugs powder (Table 3) treated with acids and alkali gave reddish brown and green fluorescence under day light and various shades of green fluorescence under UV light. Various extracts revealed characteristic yellow and green fluorescence under UV light and day light.

**TLC PROFILES**

TLC Profiles of the chloroform extract of the plant drug (Figure 6) on Silica gel "G" plate using chloroform as mobile phase and 50% Sulphuric acid as spraying reagent revealed 5 spots (Table 5) with Rf 0.15(Yellow), Rf 0.18(Green), Rf 0.28(Light blue), Rf 0.43(Brown), Rf 0.88(Purple).

*Important chemical constituents*

Flavones, Sterols and Terpenes

**CONCLUSION**

Botanical and Analytical standards of the Dodonaea viscosa (L.) Jacq. determined in the present work can contribute significantly towards the authentication of this traditional drug source which is used as an antipyretic, anti-inflammatory and wound healing agent. Such scientific data can help in the authentication and identification of traditional plant drugs which exist mostly in dry condition in the raw drug market and as pieces of bark, wood, leaf or as fragmentary materials.

The data obtained in the present work could be made use of in Ayurveda and Siddha Pharmacopoeias and can be much useful in identifying the botanical drugs unequivocally. This is the first report on the botanical and analytical standards of the Siddha Alchemy Drug source.

**REFERENCES**