A Comparative Study On Antidiabetic Effects Of Ethanol Extract Of *Origanum majorana* And *Indigofera linnaei* Ali On Streptozotocin Induced Diabetic Rats

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ABSTRACT
The evaluation of plant products on the basis of medicinal and therapeutic properties forms a platform for the discovery of newer drug molecules from different plant sources. To compare anti-diabetic effect between two ethanolic extracts of selected plants locally available in Bobbili region, Vizianagaram district, Andhra Pradesh, India on Streptozotocin induced diabetic rats. To compare anti-diabetic study, experimental rats were divided into five groups viz. Group I (Control), Group II (Diabetic, Streptozotocin, 50mg/kg bwt, i.p.), Group III (Diabetic with Glibenclamide(4mg/kg)), Group IV (Diabetic with *Origanum majorana*) and Group V (Diabetic with *Indigofera linnaei* Ali). Both plants extracts were supplemented with same dose i.e. 100mg/kg b.wt, orally. The blood glucose levels, lipid profile, body weight were evaluated in all above experimental groups before and after diabetes induction in 21 days pharmacological evaluation. Significantly decreased blood glucose level and simultaneously improved lipid profile and body weight in Group IV rats after oral administration of ethanol extract whereas in Group V rats are showing less effect compare to Group IV rats. Both the plant extracts effect compare with Group II and Group III rats statistically. *Origanum majorana* shows better antidiabetic activity because the leaf contain huge amount of antidiabetic phytoconstituents like rutin and quercetin flavonoids. In *Indigofera linnaei* Ali whole plant ethanol extract also shows antidiabetic activity because it contains kaempferol like important antidiabetic active constituents. *Origanum majorana* and *Indigofera linnaei* Ali plants may be used as a dietary supplement in diabetic patients. Further study is required to evaluate the antidiabetic activity.

**Keywords:** *Origanum majorana, Indigofera linnaei* Ali, Diabetes, Phytoconstituents

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INTRODUCTION

*Origanum majorana* (Marjoram) is an aromatic herb in the mint family (Lamiaceae). It has many medicinal uses with huge health benefits \[1\]. Digestive benefits like increasing the efficiency of digestion by increasing digestive enzymes and saliva, improving appetite, relieving nausea, eliminating flatulence, preventing intestinal infections, relieving diarrhoea and constipation \[2\]. Marjoram is a great antiseptic, antibacterial, antifungal, and antiviral agent and used in a variety of common illnesses such as food poisoning, staph infection, tetanus infection in wounds, typhoid, malaria, influenza, common cold, mumps, measles. Another benefit of marjoram is the enhancement of the cardiovascular and circulatory system like lowering the blood pressure, greatly reducing the risk of hypertension, preventing the buildup of cholesterol. Anti-inflammatory effects like asthma, muscle spasms, sinus headaches, migraines, fever, body aches etc \[3,4\]. Topical application it is for painful joints, sore muscles, sprains, back aches, toothaches4. For emotional and neurological benefits such as relieving insomnia, reducing stress, calming anxiety, minimizing emotional reactions, increasing control of sexual desire etc \[5\].

*Indigofera linnaei Ali* (Fabaceae), commonly known as Birdsville indigo \[6\]. *Indigofera linnaei Ali* plant parts of root, stem and leaves are bitter in taste is selected due to ethnopharmacological information that the plant has been used to treat anthelmintic, tonic, skin disorders, for toothache, ulcer, solid tumours, insect stings and snake bites, epilepsy, anti-nociceptive, analgesic and anti-inflammatory, antioxidant, rheumatism, arthritis, antimicrobial, antidysslipidemic, anti-fertility, antiscorbutic, diuretic, jaundice and to treat burns, liver disease and psychiatric illness, promoting growth of hair, chronic bronchitis, asthma, hydrophobia, in gastropathy and also used as thermogenic, laxative, expectorant etc \[7,8,9\].

These herbs contains important phytoconstituents like tannins, glycosides, terpenes, flavonoids, linalool and cavacrol. *Origanum majorana and Indigofera linnaei Ali* shows many medicinal effects which are useful for treatment of different common diseases \[10\]. Quercetin,Rutin, Kaempferol like flavonoids are present in these ethanolic extracts which may responsible for antidiabetic and hypolipidemic activities \[11,12\].

MATERIALS AND METHOD

Collection and Identification of Plants

The fresh *Origanum majorana* leaves and *Indigofera linnaei Ali* whole plant were collected in the month of October 2015 from the forest of Bobbili region, Vizianagaram district, Andhra Pradesh,
India, authenticated by Dr. Madhava Chetty, Department of Botany, S.V. University, Chittor Dist. Tirupati. The leaves were deposited in the Herbarium of Department of Botany.

**Preparation of Plant Extracts**

Plant materials were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried then made into fine powder. These powdered samples (100g/500ml) in ethanol for 48 hours at 45°C. The Phytochemical constituents are extracted by using Soxhlet apparatus. The extracts were soaked and evaporated under pressure and concentrated at 50°C and the residue obtained was stored at 4°C.

**Preliminary Phytochemical Screening**

Specific qualitative tests were performed to identify bioactive compounds such as tannins, alkaloids, saponins, flavonoids, terpenoids and phenols were determined from ethanol extracts of *Origanum majorana and Indigofera linnaei Ali*

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Phytochemical constituents</th>
<th>Ethanol extract of <em>Origanum majorana</em></th>
<th>Ethanol extract of <em>Indigofera linnaei Ali</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Resins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Phenols</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Cardiac glycosides</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>10</td>
<td>Terpenoids</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

**Department, Hyderabad**

The animals used in experiment were procured from animal house of Nalla Narasimha Reddy Education Society’s Group of Institution, from Pharmacy Department. Wistar rats of either sex weighing about 160–200 g were taken. Experimental protocols were approved by the Institutional Animal Ethic Committee (CPCSEA NO.-282/P0/Bt/S/2000). The animals were kept in polycarbonate cages and maintained under standard housing conditions of temperature (22 ± 20C) and humidity (45–60%) with 12 h light-dark cycle. Animals were fed pellet diet with supply of water *ad libitum* and normal saline. Animals were divided into five different groups as normoglycemic control, diabetic control, reference group, and test groups.

**Acute Oral Toxicity Study**

Acute oral toxicity studies of ethanol extracts of *Origanum majorana* and *Indigofera linnaei Ali*
were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD) no. 423. As per OECD guidelines minimum number of animals should be used (3 animals per dose) for experiment to obtain the information as acute toxicity of test dose \[^{13}\]. Overnight fasted rats were orally fed with plant extract at a dose level of 250, 500, 1000 and 2000 mg/kg body weight respectively. The animals were observed continuously for 2hrs to investigate any sign of toxicity, occasionally for 4 hrs for their general behavior and after a period of 24 hr, animals were observed for any sign of mortality till 7 days \[^{14}\].

**Induction of diabetes experimentally**

Wistar rats (160-200gm) were fasted for 18 hours before the induction of diabetes with Streptozotocin (STZ), for induction of Type-1 Diabetes mellitus. Animals (n=30) were injected intraperitoneal with 0.22-0.25ml of freshly prepared solution of STZ (50 mg/ml in 0.01 m citrate buffer, pH 4.5) at a final dose of 50 mg/kg body wt. The diabetic state was assessed in STZ-treated rats by measuring the non-fasting serum glucose concentration after 72 hours. Only rats with serum glucose levels greater than 200-250 mg/dl were selected and used in this experiment.

**Experimental Design for Oral Glucose Tolerance Test (OGTT)**

In oral glucose tolerance test, animals of diabetic control group have shown significant elevation in blood glucose level through entire study when compare to normal animals. But treatment with standard drug glibenclamide and ethanol extracts (100 mg/kg) of *Origanum majorana* and *Indigofera linnaei Ali* could able to reduce significantly \(P<0.01\) blood glucose level in therapeutic groups after 60 mins and 120 mins \[^{15, 16}\]. The results of OGTT have shown in [Table No 2].

**Streptozotocin-induced Diabetic Model**

The animals were divided into five groups of six rats each. The ethanolic extracts were administered for 21 days. Group I served as normal control rats administered sodium carboxy methyl cellulose (SCMC) daily for 21 days; Group II diabetic control rats administered STZ (50mg/kg) with SCMC; Group III diabetic rats administered standard drug glibenclamide (4 mg/kg); Group IV diabetic rats administered *Origanum majorana* (100 mg/kg); Group V diabetic rats administered *Indigofera linnaei Ali* (100 mg/kg). The fasting glucose levels were determined on days 1, 7, 14 and 21 of extracts administration. During the experimental period, the blood glucose level, the lipid level and body weight of different group animals are estimated \[^{17, 18}\].

**Estimation of Biochemical Parameters**

The biochemical parameters were determined on day 21 after the animals were sacrificed by cervical dislocation. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL)
and low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) were determined by the glucose oxidase method, using an auto-analyzer [19, 20].

**Statistical Analysis**

Results of estimation of biochemical and functional parameters have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet’s test (Sigma stat 3.5). P values <0.05, were considered statistically significant.

**RESULTS AND DISCUSSION**

**Preliminary Phytochemical Screening:**
Specific qualitative tests identified the phytochemicals constituents such as tannins, alkaloids, saponins, flavonoids, terpenoids, and phenols from ethanol extracts of *Origanum majorana* and *Indigofera linnaei Ali*.

**Acute Oral Toxicity Study:**
The result of acute toxicity study of ethanol extracts of above plants on laboratory animals showed that no lethality up to the dose of 2000 mg/kg body weight hence the animals were safe up to a maximum dose of 2000 mg/kg body weight.

**Oral Glucose Tolerance Test (OGTT)**
The effects of ethanolic extracts of selected plants on the plasma glucose level are illustrated in table 2. Both ethanolic extracts showed significant reduction in plasma glucose level in rats at 90 minutes, and same was observed in standard drug at 60mins. 100 mg/kg body weight of both plants extracts treated rats produces significant reduction in plasma glucose level, while in disease control rats, plasma glucose level was increased.

**Effect of Ethanolic Extract on Streptozotocin-induced Diabetic Rats:**
The diabetes rats were confirmed with increasing level of fasting plasma glucose level. The effect of ethanol extracts at same dose (100mg/kg) of *Origanum majorana* and *Indigofera linnaei Ali*, on fasting plasma glucose level of normal and streptozotocin induced are given in table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels were statistically significant by compare with diabetic rats.

**Effect of Ethanolic Extract on Biochemical Parameters in Streptozotocin-induced Diabetic Rats:**
Both plants ethanolic extracts on diabetes induced hyperlipidemia were also evaluated. It was observed that due to diabetes there was an increase in the total cholesterol levels as well as
triglyceride levels. The HDL levels were reduced in the diabetic animals and the LDL levels were increased significantly (Table 4). The ethanol extracts showed a significant decrease in the total cholesterol levels and triglyceride levels. It also increased the HDL level and was successful in suppressing the LDL and VLDL levels as compared to the standard drug (Table 4). Compared to both plant extracts *Origanum majorana* shows better effect compared to *Indigofera linnaei* Ali.

**Table 2: Effect of ethanolic extracts of *Origanum majorana* (EEOM) and *Indigofera linnaei* Ali (EEIL) (100 mg/kg, PO), on oral glucose tolerance test (OGTT) in normal and streptozotocin induced diabetic rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment of Dose(mg/kg)</th>
<th>BLOOD GLUCOSE LEVEL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fasting</td>
</tr>
<tr>
<td>I</td>
<td>Normal control(Normal Saline)</td>
<td>77.5±0.63</td>
</tr>
<tr>
<td>II</td>
<td>Disease Control(STZ induced)</td>
<td>86±0.88</td>
</tr>
<tr>
<td>III</td>
<td>Diabetes+Glibenclamide(4mg/kg)</td>
<td>83.5±0.82</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetes+EEOM(100mg/kg)</td>
<td>85.6±0.71</td>
</tr>
<tr>
<td>V</td>
<td>Diabetes+EEIL(100mg/kg)</td>
<td>88.25±0.63</td>
</tr>
</tbody>
</table>

Values are given as Mean ± SEM for n=6. Group IV was compared with group I and II. Group IV and V were compared with group II. Values are statistically significant at **p< 0.01.**

Ethanol extract of *Origanum majorana*, EEIL-Ethanol extract of *Indigofera linnaei* Ali

![Oral glucose tolerance test (OGTT) in normal and streptozotocin induced diabetic rats.](image)

**Figure 1: Effect of *Origanum majorana* and *Indigofera linnaei* ethanol extracts on OGTT in normal and diabetic rats.**
Table 3: Effect of multiple dose treatment of ethanolic leaf extract of *Origanum majorana* and *Indigofera linnaei* Ali (100 mg/kg, PO), (Once daily), on blood glucose level after 21 days in normal and Streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Treatment of Dose (mg/kg)</th>
<th>Basal value</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control (Normal Saline)</td>
<td>78.23±0.73</td>
<td>87±0.52</td>
<td>98±0.28</td>
<td>91±0.54</td>
<td>85±0.69</td>
</tr>
<tr>
<td>II</td>
<td>Disease Control (STZ induced)</td>
<td>248.57</td>
<td>252.5±2.15</td>
<td>259.8±1.89</td>
<td>263.5±2.24</td>
<td>266.25±2.71</td>
</tr>
<tr>
<td>III</td>
<td>Diabetes+Glibenclamide (4mg/kg)</td>
<td>240.36±2.29</td>
<td>228.52±2.14*</td>
<td>154.2±0.16</td>
<td>130.05±1.29</td>
<td>101±1.412</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetes+EEOM (100mg/kg)</td>
<td>244.37</td>
<td>235.15±2.85*</td>
<td>162.8±2.45</td>
<td>140.25±1.24</td>
<td>125.5±0.54</td>
</tr>
<tr>
<td>V</td>
<td>Diabetes+EEIL (100mg/kg)</td>
<td>247.25±2.67</td>
<td>241.45±2.71*</td>
<td>196.57±2.38</td>
<td>165.05±1.85</td>
<td>136.25±1.91</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P*<0.05, when compared with diabetic control group.

Figure-2: Effect of *Origanum majorana* and *Indigofera linnaei* ethanol extracts on blood glucose level in diabetic rats.
Table 4: Effect of ethanolic leaf extract of *Origanum majorana* and *Indigofera linnaei Ali* (100 mg/kg, PO) on Serum Biochemical Parameters after 21 days treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>TC</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>136±1.89</td>
<td>43.25±0.75</td>
<td>92±0.81</td>
<td>16.23±0.62</td>
<td>72.84±0.96</td>
</tr>
<tr>
<td>II</td>
<td>Disease control (STZ induced)</td>
<td>184.6±0.91</td>
<td>20.17±0.39</td>
<td>109.7±1.62</td>
<td>18.89±0.56</td>
<td>98±2.86</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic+ glibenclamide (4mg/kg)</td>
<td>151.05±1.49</td>
<td>44±1.25</td>
<td>82.55±2.92</td>
<td>17.65±0.37</td>
<td>84.2±1.55</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + EEOM (100mg/kg)</td>
<td>169.23±1.24</td>
<td>37.18±1.39**</td>
<td>96.40±1.65*</td>
<td>22.25±0.35**</td>
<td>93.2±2.36*</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + EEIL (100mg/kg)</td>
<td>174.05±1.56</td>
<td>35.07±1.41</td>
<td>99.62±1.55</td>
<td>19.42±0.25</td>
<td>95.37±2.27</td>
</tr>
</tbody>
</table>

The values indicate mean ±S.E.M (n=6). p<0.05 compared with normal control values and p<0.05 compared with diabetic control values and test drugs.

**DISCUSSION**

From qualitative preliminary phytochemical screening revealed that alkaloids, flavonoids, tannins, glycosides, sterols, phenols, terpenoids, steroids like phytoconstituents are present in both the plants ethanol extracts. It can be depicted from the observations of OGTT results (Figure. 1), that...

![Lipid profile after 21 days drug treatment on normal and diabetic rats](image-url)
ethanol extract of *Origanum majorana* leaves reduced greater glucose concentration, where as *Indigofera linnaei Ali* shows less response comparatively. The results of former plant extract were quite interesting. *Origanum majorana* leaves extract showed a significant decrease in the blood glucose level after 7th day treatment. Interestingly, *Indigofera linnaei Ali* which shows less response during OGTT, but later significant decrease in the blood glucose level also which indicating anti diabetic potential on prolonged treatment. However, the percentage reduction in the blood glucose level is less than that treated with *Origanum majorana* leaves extract (Figure 3). It was investigated that the phytoconstituents such as flavonoids, saponins and triterpenes are beneficial to control diabetes. The qualitative analysis of *Origanum majorana* and *Indigofera linnaei Ali* ethanol extracts confirm the presence of such active phyto components. The flavanoids play a major role in maintaining blood glucose levels and lipid profiles in diabetes condition. The consumption of flavanoids or flavanoid rich foods reduces the risk of diabetes mellitus. It was reported that Rutin, quercetin and eridictyol like isolated flavonoid components are present in leaves extract of *Origanum majorana* which shows positive antidiabetic and hypolipidemic activities. In case of *Indigofera linnaei Ali*, it is also shows antidiabetic activity because of Kaempferol and quercitin like flavonol active constituents are present which maintain blood glucose level in diabetic condition. In the above study *Origanum majorana* ethanol leaves extract comparatively shows better response then whole plant ethanol extract of *Indigofera linnaei Ali*. On the other hand, Saponins also present in both the plants and it have the ability to reduce increased plasma blood glucose level hence; it may useful in treatment of diabetes mellitus. Similarly, the presence of terpenoids inhibit enzymes involved in glucose metabolism and prevent the development of insulin resistance thus normalising insulin levels. A marked increase in serum concentration of TC, LDL, VLDL, TG and decreased HDL was observed with diabetic rats than normal control group which is generally known as hyperlipidaemia. From this study it was reveals that the administration of EEOM and EEIL not only lowered TC, LDL, VLDL and TG but also enhanced the cardioprotective lipid HDL.

**CONCLUSION**

The antidiabetic study reveals that *Origanum majorana* leaves has significant anti-diabetic potential and the ethanol leaves extract of this plant may be used as herbal medicine which is substitute for synthetic drugs to treat diabetic patients. On the other hand prolonged treatment with *Indigofera linnaei Ali* whole plant ethanol extract showed reduction in the blood glucose level and
maintain lipid profile, it can also be used as an anti-diabetic drug with lesser potential than *Origanum majorana*.

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