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## Comparative antioxidant activity of *Gymnema sylvestre*, *Enicostemma littoral*, *Momordica charantia* and their composite extract

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### Abstract

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases, diabetes and inflammatory diseases. The current paper evaluates the antioxidant potential of *Gymnema sylvestre* (leaves), *Enicostemma littorale* (aerial parts), *Momordica charantia* (fruits) extracts and their composite extract. The present study resulted a potent antioxidant composite extract with IC<sub>50</sub> of 45 µg/ml. The individual plant extracts shows moderate antioxidant activity. Combining of plants resulted enhanced antioxidant activity, thus the efficacy of composite extract was found compared with standard synthetic antioxidant compounds (BHA and Vitamin C). The current study leads in a development of herbal composite extract from widely reported herbal drugs *Gymnema sylvestre*, *Enicostemma littorale* and *Momordica charantia* for the effective antioxidant agent. The developed composite extract can be used as natural antioxidant that is side effect-free alternative to synthetic antioxidants in the food processing industry and for use in preventive medicine.

**Keywords:** Antioxidant activity, *Gymnema sylvestre*, *Enicostemma littorale*, *Momordica charantia*, DPPH.

### 1. Introduction

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in plants [1]. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are currently used as food additives, and many plant species have similar antioxidant potentials as these synthetics. *Gymnema sylvestre* R.Br. (Asclepiadaceae) is an herb native to the tropical forests of southern and central India and Sri Lanka. Chewing the leaves destroys the ability to discriminate sweet taste, giving its common name, *Gur-mar*. Traditionally it is used as antidiabetic, hypolipidemic, stomachic, diuretic, refrigerant, astringent and tonic. The major bioactive constituents of *G. sylvestre* are a group of oleanane type-triterpenoids known as gymnemic acid [2]. Gymnemic acid has been reported as antihyperglycemic, normoglycemic and antihyperlipidemic in *in-vitro* studies [3, 4]. Gymnemic acid elicit antihyperglycemic by increasing serum insulin levels due to regeneration of pancreatic cells [5], stimulating insulin release [6] and inhibition of glucose absorption [7].

*Enicostema littorale* Blume locally known as *Mamejava* or *Chota-chiretta*, is commonly used Ayurvedic medicine for treatment of diabetes. It is a glabrous perennial herb and found throughout India [8]. The plant is known for several medicinal uses like antidiabetic, hypolipidemic, antipyretic and anti-inflammatory activities. It is also reported to have anti-inflammatory [9], anticancer [10], hypoglycemic, antioxidant, hypolipidemic and insulinotropic [11] activities. Antidiabetic effects of *E. littorale* were also reported by other workers [12-14]. Swertiamarin is major bioactive compound present in *Enicostema* and it is reported to have antihyperlipidaemic [15, 16], hypoglycemic, insulinotropic [17, 18] and antinociceptive [19] activities. Swertiamarin and its metabolite, gentianine have anti-diabetic effect [20] besides normalizing altered lipid profile associated with diabetes [21]. *Momordica charantia* Linn. belongs to family Cucurbitaceae and its fruits are commonly known as Bitter melon. Its fruits and seeds are widely reported to have antidiabetic, antihyperlipidaemic, anticancer, anti-HIV, anti-ulcer, anti-tumor, antiviral, analgesic, anti-inflammatory, hypotensive, anti-fertility,

hepatoprotective and antioxidant activities [22]. *M. charantia* fruits reduced blood glucose levels, improved body weight and glucose tolerance [23, 24]. Its extract decreased insulin resistance in rats fed on high-fructose diet [25], increased the mass of  $\beta$ -cells and enhanced insulin production in pancreas [26]. Charantin is the main active constituent of *M. charantia* fruits and consists of a mixture of steroidal glycosides, namely sitosteryl glucoside and stigmasterol glucoside [27]. The aim of present study was to develop an herbal composite extract from herbal drugs viz. *G. sylvestre*, *E. littorale* and *M. charantia* for the effective antioxidant agent.

## 2. Materials and Methods

### 2.1. Plant Materials

Authentic drug samples of *Gymnema sylvestre* (leaves), *Enicostemma littorale* (aerial parts) and *Momordica charantia* (fruits) were procured from crude drug market. The authenticity of all the accessions was ascertained by Taxonomists and the voucher specimens were preserved for future reference.

### 2.2. Chemicals

BHA (3-tert-butyl-4-hydroxyanisole), Vitamin C and DPPH (2,2-dyphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich, Bangalore, India. All other solvents and chemicals were of analytical grade.

### 2.3. Preparation of Extract

The *G. sylvestre* (leaves), *E. littorale* (aerial parts) and *M. charantia* (fruits) were dried in an oven at 45 °C. Dried drug samples were pulverized to a coarse powder using a grinder. The powdered sample of drugs (10 g) was extracted in Ultrasonicator for 30 min with ethanol (250 ml). The extracts were filtered and evaporated under reduced pressure at 50 °C in a rotary evaporator (Buchi, Switzerland). The composite extract was prepared by mixing equal quantities of *G. sylvestre*, *E. littorale* and *M. charantia* extract.

### 2.4. Evaluation of antioxidant (DPPH scavenging) activity

The ability of *G. sylvestre* (leaves), *E. littorale* (aerial parts) and *M. charantia* (fruits) and their composite extract to scavenge DPPH free radicals were assessed by the standard method [28, 29]. Briefly, the ethanolic extracts of *G. sylvestre* (leaves), *E. littorale* (aerial parts) and *M. charantia* were reconstituted in methanol. The stock solution of all extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions 1000, 500, 250, 125, 62.5, 31.25, 15.62 and

7.81  $\mu$ g/ml were prepared by serial dilution method. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH (1 mg/ml). After 30 min incubation in darkness at room temperature (25 °C), the absorbance was recorded at 517 nm. Control sample contains all the reagents except the plant extract. BHA and Vit. C was used as standards for comparison. Percentage inhibition was calculated using equation given below:

$$\% \text{ Inhibition} = \frac{A_{co} - A_t}{A_{co}} \times 100$$

Where,  $A_{co}$  is absorbance of the control and  $A_t$  is absorbance of the samples.

$IC_{50}$  values were estimated from the % inhibition versus concentration plot using a non-linear regression algorithm.

## 3. Result and discussion

The antioxidant activities of *G. sylvestre* (leaves), *E. littorale* (aerial parts) and *M. charantia* (fruits) and their composite extract were determined by using a methanolic solution of DPPH. The results of antioxidant activity were expressed in terms of percentage of inhibition (%). Parallel to examination of the antioxidant activity of the plants extracts individually and in their combination, the values for two standard compounds (BHA and Vitamin C) were obtained and compared. A plot of % inhibition versus concentration given in Figure 1 was used to calculate  $IC_{50}$  values.

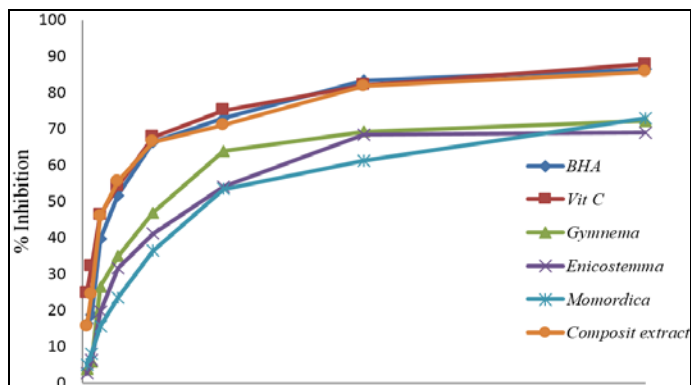
The results of antioxidant activity were summarized in Table 1. The examination of antioxidant activity of *G. sylvestre* (leaves) showed concentration dependant response and it varied from 3.92 to 72.22% for 7.81 to 1000  $\mu$ g/ml, respectively (Figure 1). The  $IC_{50}$  values of *G. sylvestre* extract was found to be 140  $\mu$ g/ml. From the Table 1, it is clear that the *G. sylvestre* extract posse's antioxidant activity that may be responsible for its beneficial effects in diabetes. The antioxidant activity of ethanolic extract of aerial parts of *E. littorale* showed concentration dependant response and it varied from 2.61 to 69.08% for 7.81 to 1000  $\mu$ g/ml, respectively (Figure 1). The  $IC_{50}$  values of *E. littorale* extract was found to be 205  $\mu$ g/ml. The antioxidant activity of ethanolic extract of *M. charantia* (fruits) showed concentration dependant response and it varied from 5.20 to 73.01% for 7.81 to 1000  $\mu$ g/ml, respectively (Figure 1). The  $IC_{50}$  values of *E. littorale* extract was found to be 225  $\mu$ g/ml.

**Table 1:** Antioxidant activity of *G. sylvestre*, *E. littorale*, *M. charantia* and their composite extract

Conc. ( $\mu$ g/ml)	BHA	Vit. C	<i>G. sylvestre</i>	<i>E. littorale</i>	<i>M. charantia</i>	Composite extract
7.81	16.38	24.73	3.92	2.61	5.20	15.53
15.62	18.86	32.31	5.88	6.20	8.21	24.68
31.25	39.67	46.45	26.66	19.60	15.68	45.99
62.5	51.84	54.39	34.99	31.69	23.63	55.74
125	66.34	67.78	46.92	41.17	36.41	66.52
250	72.97	75.16	64.05	54.18	53.42	71.09
500	83.39	82.17	69.28	68.43	61.36	81.87
1000	86.51	87.90	72.22	69.08	73.01	85.64
$IC_{50}$ values	55	45	140	205	225	45

The antioxidant activity of composite extract of *G. sylvestre* (leaves), *E. littorale* (aerial parts) and *M. charantia* (fruits) showed concentration dependant response and it varied from 15.53 to 85.64% for 7.81 to 1000  $\mu$ g/ml, respectively (Figure

1). The  $IC_{50}$  values of *E. littorale* extract was found to be 45  $\mu$ g/ml. From the Figure 1, it was clear that the composite extract shows strong antioxidant activity and scavenges the DPPH higher than the individual plants.



**Fig 1:** Antioxidant activity of *G. sylvestre*, *E. littorale*, *M. charantia* and their composite extract

The IC<sub>50</sub> values of standard BHA and Vitamin C was found to be 55 µg/ml and 45 µg/ml, respectively. On the basis of IC<sub>50</sub> values of standard compounds (BHA and Vitamin C) and composite extract, the antioxidant potential of composite extract was found superior to BHA and comparable to Vitamin C. Thus the developed composite extract may be used as potential antioxidant herbal drug.

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