AMERICAN JOURNAL OF PHARMTECH RESEARCH

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Pharmacognostical and *In-vitro* Antioxidant Study of Indian Antidiabetic Plants

Kaushik B. Kanada[1], N. M. Patel[2], M. M. Patel[1]

1. Shankersinh Vaghela Bapu Institute of Pharmacy, Vasan, Gandhinagar, Gujarat.

2. Laxminarayan Dev college of Pharmacy, Bholav, Bharuch, Gujarat.

ABSTRACT

Aim of this study is to give an enlightment on pharmacognosy and in-vitro antioxidant activity of Among them *E. littorale* leaf, *G. sylvestre* leaf and *M. charantia fruits* which are used as antidiabetic drug in India since ancient time. These plants traditionally used in diabetes, liver disorders, fever and inflammation. The antioxidant activity was measured by DPPH method.

Keywords: Anti oxidant, *E. littorale*, *E. littorale* leaves, *G. sylvestre* leaf and *M. Charantia* fruits

*Corresponding Author Email: kaushikkanada@rediffmail.com

Received 12 November 2014, Accepted01 Decemebr 2014

Please cite this article as: Kanada KB *et al*., Pharmacognostical and *In-vitro* Antioxidant Study of Indian Antidiabetic Plants. American Journal of PharmTech Research 2014.

INTRODUCTION

Medicinal plants have been used as sources of medicine in virtually all cultures[1]. They are of important therapeutic aid for various ailments. During the last decade, the use oftraditional medicine has expanded globally and its gaining property. It has continued to be used in not only for primary health care of the poor in developing countries, but also in countries where conventional medicine in predominant in the national health care system[2]. According to the WHO, herbal medicines serve the health needs of about 80%of the world's population, especially for millions of people in the vast rural areas of developing countries[3].

***Enicostemma littorale* Blume** Drug consists of fresh or dried whole plans of (mostly leaves and roots) of *Enicostemma littorale* Blume (*Enicostemma hyssopifolium* Willd.), belongs to family Gentianaceae. The word *Enicostema* is probably formed from the three words *en* (in, inside), *icos* (20), and *stemma* (wreath, circle), due to the many flowers arranged in circles in the leaf axils along the stem. The plant is also known as Mamejava, mamjjak, chota-chirayata, Kada-vinayi, manucha. It is erect perennial herb, 5-30cm. tall, simple or branched at the base, longer than the internodes, glabrous, distributed throughout India including coastal region up to an altitude of about 450m[4].

***Gymnema sylvestre* (Retz.) Schult.** It consists of fresh or dried leaves of *Gymnema sylvestre* (Retz.) Schult. family Asclepidaceae. It is also known as *Gymnema melicida, Periploca sylvestris, Asclepias geminate,* Periplora of the wood, Meshashringi, Sarpadarushtrika (in Sanskrit), merasingi, gurmar, ghhota-dudhilata, Kavali, Dhuleti30. It is habitat of Central and Western peninsula and near the coast at Karwar, North Kanara, Bandelkhand, Saharanpur, Bihar, N. Circars, Deccan and Carnatie in dry forests, up to about 2,000 ft in the hills31. It is a large, woody, much branched climber with pubscent young parts having simple, opposite, elliptic or ovate, more or less pubescent on both sides, cordate at base leaves[5,6].

The drug consists of fresh fruits of *Momordica charantia* Linn. belongs to family Cucurbitaceae. It is monoecious, herbaceous climber cultivated throughout India up to an altitude of 1500m[7]. The plant is also known as Bitter Gourd, Karavella, Sushavi, Karela, Kareli and Kurela-Jangro48 Karela plant is widely grown in tropical and subtropical regions of the world such as India, Asia, and South America. Immature fruit is green, elongated, fusiform, longitudinally grooved, ridged and warty, 2.5-25 cm long, 2-7cm in diameter, pulp pithy, whitish yellow at maturity, splittin into 3 components, exposing numerous seeds, enclosed in white aril which becomes bright red on maturity77. Odour: characteristic, Taste: Bitter.

MATERIALS AND METHOD

Herbal materials are categorized according to sensory, macroscopic and microscopic characters. Study of morphology and microscopic characters is the first step to establish identity and the degree of purity of the materials, and should be carried out before undertaking any tests. Macroscopic identity of herbal materials is based on the study of shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface. However, these characters are judged subjectively and substitutes or adulterants may closely resemble to the genuine material, hence it is often necessary to substantiate the findings by microscopy and/or physicochemical analysis. Cellular and tissue arrangement is specific in all individual plants. Microscopic inspection of herbal materials is indispensable for the identification of broken or powdered materials; the specimen may have to be treated with chemical reagents. Microscopic examination alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence. Macroscopic and microscopic study of the leaves of *E. littorale* and *G. sylvestre* and fruit of *M. charantia* were carried out by following methods[8].

Morphological study[9]

- **Size:** Length, width and thickness of crude materials were measured by a graduated ruler in millimeters.
- **Colour:** The colour of the untreated sample was examined under diffuse daylight and should be compared with that of a reference.
- **Surface characteristics, texture and fracture characteristics:** Untreated sample was examined with magnifying lens (6X to 10X). If needed, it was wetted with water or reagents, to observe the characteristics of a cut surface. The material was touched to determine softness or hardness; bended and ruptured it to obtain information on brittleness and the appearance of the fracture plane, whether it is fibrous, smooth, rough, granular, etc.
- **Odour:** Small portion of the sample was placed in the palm of the hand or in a beaker of suitable size, and slowly and repeatedly inhaled the air over the material and determined the strength of the odour (none, weak, distinct, strong) and then the odour sensation (aromatic, fruity, musty, mouldy, rancid, etc.).

Microscopic evaluation[10]

- **Procedure:** Once the material has been examined and classified according to external characteristics, inspection by microscopy was carried out as the next step. Microscope was

equipped with lenses with wide range of magnification, a substage condenser, graduated mechanical stage, objectives lenses with magnification of 4X, 10X and 40X, a set of drawing attachments for the microscope; slides and cover slip of standard size and a set of botanical dissecting instruments were required for microscopic examination.

- **Preliminary treatment:**

  A dried plant part representing the sample material was softened before preparation for microscopy, preferably placing in a moist atmosphere or soaking in water. Boiling in water for a few minutes was necessary for the leaf part.

- **Powdered materials:**

  Place 1 or 2 drops of water, glycerol/ethanol or chloral hydrate solution on a glass slide. Moistened the tip of a needle with water and dipped into the powder. Transferred a small quantity of the powder material adhered to the needle tip into the drop of fluid on the slide. Heated the slide on burner and Stir thoroughly, but carefully, and apply a cover-glass. Pressed lightly on the cover-glass with the handle of the needle, and removed excess fluid from the margin of the cover-glass with a strip of filter-paper.

- **Surface tissues of leaves:**

  The leaves were boiled directly on a slide to render pieces of thin transparent leaves. Cut a piece of leaf into two portions, turned one piece upper side down and added chloral hydrate solution. The surface of the two portions scraped using a scalpel until only a single layer of epidermis remained and was washed with drops of chloral hydrate solution or glycerol/ethanol to remove any residues.

- **Transverse Sections:**

  The part of plant representing the material was selected and cut into suitable lengths, one end of which is softened and cut into thin section either in a radial direction (Transverse section) or in a tangential direction (Longitudinal section).

The dried plant materials were evaluated for macroscopic and microscopic characters like; shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface, cellular and tissue arrangement of the *Enicostemma littorale* leaves, *Gymnema sylvestre* leaves and *Momordica charantia* fruit.

***In vitro* Antioxidant Activity[11]**

In vitro Anti-oxidant activity of hydroalcoholic extract of *E. Littorale* leaves, *M. Charantia* fruits and *G. Sylvestre* Leaves were carried out using DPPH radicals scavenging activity and ferric reducing power ability (FRPA) assay models.

DPPH radicals scavenging activity

DPPH solution (150µl) was added to 3ml water and absorbance was taken after 30min. at 516nm for control reading. Different concentrations (200-1000µg/ml) of Hydro-alcohol extract of *E. Littorale* leaves, *G. Sylvestre* leaves and *M. Charantia* fruits and reference standard ascorbic acid (10-100µg/ml) in methanol were mixed with 150µl DPPH and diluted up to 3ml with water. The mixture was kept in dark for 30min. and absorbance was measured at 516nm after 30min. The % reduction was calculated as follow.

$$\textbf{\% Scavenging Activity} = \frac{A_B - A_A}{A_B} \times 100$$

Where; $\mathbf{A_A}$ is the absorbance of the tested sample after 30 minutes.

$\mathbf{A_B}$ is the absorbance of Control sample.

IC_{50} represents the concentration of a drug that is required for 50% inhibition.

RESULTS AND DISCUSSION

***Enicostemma littorale* leaves**

Morphology

Leaves of *E. littorale* were found green, lanceolate and sessile, Apex of the leaf was obtuse, margin was entire, venation was pinnate, lamina was 3-6X0.5-0.7Cm, base was symmetrical and upper surface was rough, lower was glabrous with characteristic odour and slightly bitter taste.

Microscopy

A dried leaves of *E. littorale* was softened by boiling in water for a few minutes before preparation for microscopy. Transverse section of the *E. littorale* leaf was taken passing through midrib, stained and mounted under microscope.

Transverse section of the *E. littorale* leaf passing through midrib was almost flat on the upper surface and convexly projected on lower surface. It showed bicollateral vascular bundle in the center; chloroplasts were lying underneath on upper epidermis. In the lamina portion, the cells of the upper epidermis were bigger in size than that of lower with striated cuticle. In surface preparation of the upper epidermal cells have slightly wavy anticlinal walls than those of the lower one and was traversed with anomocytic to anisocytic type of stomata, few on upper, but many on lower side, simple unicellular trichomes were present on both the surfaces. Sessile glandular

trichomes with bicellular head were found chiefly over the veins, 5-6 rows of spongy parenchyma occupied by the mesolphyll tissue.

Powder microscopy

Powder Microscopy of E. littorale leafMicroscopic study of *E. littorale* leaves powder shown fragments of upper and lower epidermal cells with striated cuticle and anomocytic to anisocytic stomata; bearing simple unicellular trichomes, sessile glandular trichomes.

***GYMNEMA SYLVESTRE* LEAVES**

Morphology

Leaves of *G. Sylvestre* were found green, simple, opposite, elliptic or ovate, apex of the leaf was acute, margin was entire, , base was cordate and more or less pubescent on both sides with characteristic odour and acrid and tingling taste.

Microsocpy of *G. sylvestre* leaves

A dried leaves of *G. sylvestre* was softened by boiling in water for a few minutes before preparation for microscopy. Transverse section of the *G, sylvestre* leaf was taken passing through midrib, stained and mounted under microscope. Transverse section of *G. Sylvestre* leaf passing through midrib was flat on the upper surface and convexly projected on lower surface. It showed amphicribal vascular bundle in the centre; spongy parenchyma was present. The lamina was dorsiventral. In the lamina portion striated cuticle was followed by single layered epidermis. The upper epidermal cells were hexagonal while the lower epidermal cells were slightly wavy. The single layered closely arranged palisade cells were present just below the upper epidermis. The spongy parenchyma was 3-5 cells thick with large intercellular spaces.. In the surface preparation of the uniseriate multicellular trichomes were observed on the both epidermii but anomocytic stomata were seen on lower epidermis only.

Powder microscopy

Microscopic study of *G. sylvestre* leaves powder shown fragments of upper and lower epidermal cells with striated cuticle and anomocytic to anisocytic stomata; bearing simple unicellular trichomes, sessile glandular trichomes.

***MOMORDICA CHARANTIA* FRUITS**

Morphology

M. charantia fruits were found green, elongated, longitudinally grooved, ridged and warty. The size of fruit was 2.5-25cm long and 2-7cm in diameter. The pulp was pithy and when cut it was splitting into 3 components with the numerous seeds, enclosed in white aril. Seeds were pale brown up to 1.5cm long, flattened and elliptic in shape. Scalloped markings were found on the flat

side and on the edge of seed. The endosperm was thin with bulky embryo; consisted of large cotyledons and short straight radical. Characteristic odour and bitter taste was observed.

Microscopic description

A fresh fruit of *M. charantia* was softened by boiling in water for a few minutes before preparation for microscopy. Transverse section of the *M. charantia fruit* was taken, stained and mounted under microscope.

Transverse section of the *M. charantia* fruit was circular in outline with many external longitudinal rugose folds, of the outer mesocarp. This part covers the major area of the section with encircling the inner whitish, pithy, spherical mesocarp. The scattered seeds were encircled by arillus. The epidermis layer was consists of squares to rectangular thin walled cells with striated cuticle; short glandular trichomes with multicellular stalk and multicellular head were found in epidermis. Hypodermis was consists of thin walled chlorenchymatous tissue. Outer and middle mesocarp stissues were spongy and porous and contains starch grains. The cells of the inner mesocarp were smaller in size. Bicollateral vascular bundles were found throughout the mesocarp tissue; endocarp was consisted of thin walled more or less tangentially elongated cells often adhering to the seed.

Transverse section of the seed was consisted of outer epidermis of palisade cells with thick cuticle. The subepidermis was 3-4 layered of small isodiametric cells which was followed by thick walled tangentially elongated sclerenchymatous layer and below this, the spongy parenchyma was present. -. Perisperm was narrow consists of collapsed cells, one layered endosperm containing oil and aleurone grains, followed by tissue of the cotyledon.

Powder microscopy

Microscopic study of *M. charantia* fruits powder shown small glandular trichomes with multicellular stalk and multicellular head, epidermal cells, hexagonal thick walled cells of the testa, simple starch grains, palisade like cells of the epidermis of testa and spongy parenchymatous cells containing starch grains and spirally thickened xylem vessels. From the results of phytochemical screening it was observed that alcoholic and aquous/water extracts contains phenolic and tannins, tri-terpenoids, saponins and flavanoids. These types of constituents might be responsible for the pharmacological activity.

Evaluation of in-vitro Antioxidant Activity by 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging method

The free radical-scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability. The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay which is commonly employed for screening of plant extracts was used for hydrogen donating capacity. The DPPH

radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability. The plants with higher donating capacity have shown higher DPPH free radical scavenging activity. Methanolic extracts of *E. littorale* leaves, *G. Sylvestre leaves* and *M. charantia* fruits were evaluated for antioxidant activity and the results were compared with the standard value of ascorbic acid. The results were reported in terms of % scavenging and IC50 values.

Table 1: The comparison of % scavenging of methanolic extracts of *E. littorale* leaves, *G. sylvestre* leaf and *M. Charantia* fruits.

Concentration of methanolic extract (µg/ml)	Antioxidant activity (%)			
	Ascorbic Acid	E. littorale Mt. ext.	G. sylvestre Mt. ext	M. charantia Mt ext
0	0	0	0	0
200	68.54±0.55	46.65±0.23	61.32±0.11	31.67±0.97
400	81.39±0.68	52.55±0.17	70.06±0.13	37.94±0.86
600	86.76±0.29	58.9±0.12	79.37±0.15	61.19±1.11
800	90.46±0.42	65.37±0.13	84.64±0.35	69.72±0.35
1000	92.62±0.66	70.34±0.26	87.39±0.32	72.89±0.45

values represent means ± SD, n=3

Table 2: IC_{50} value of Ascorbic Acid, methanolic extract of *E. littorale* leaves, *G. Sylvestre* leaf and *M. charantia* fruits

Ingredient	IC_{50} (µg/ml)
Ascorbic Acid (Std.)	128.35
E. littorale leaves extract	240.02
G. sylvestre leaf extract	142.67
M. Charantia fruits extract	510.23

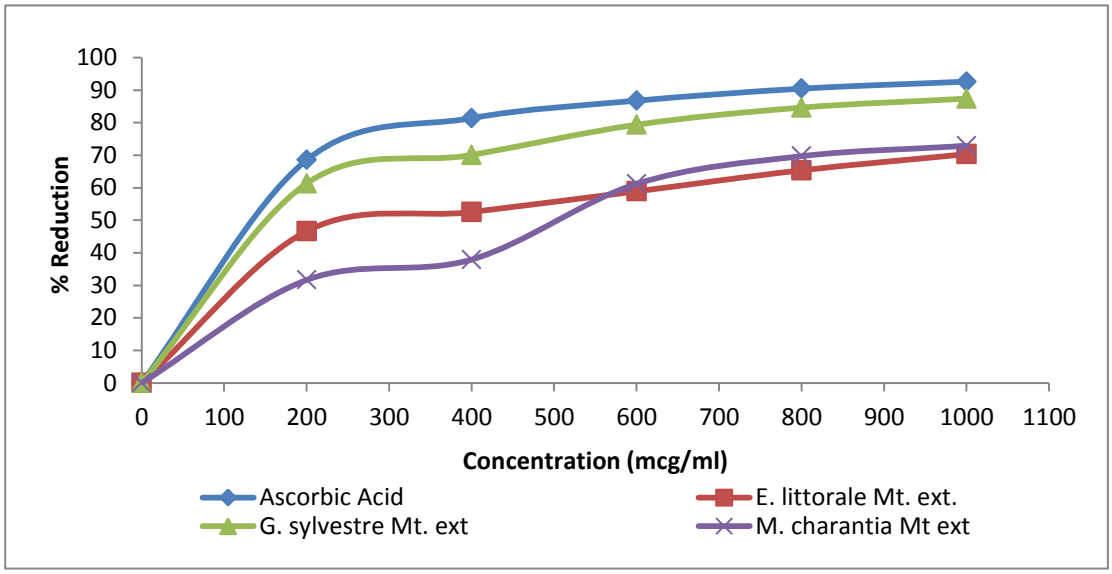

Figure 1: Data showing the comparison of % scavenging in methanolic extracts of *E. littorale* leaves, *G. sylvestre* leaf and *M. Charantia* fruits.

Table shows the results of the free radical (DPPH) scavenging activity in (%) inhibition. The result revealed that the methanolic extract of *Gymnema sylvestre* exhibited the highest DPPH radical scavenging activity with 87.39±0.32% at 1000µg/ml concentration (which is nearly close to the value of Ascorbic acid i.e. 92.62±0.66%) followed by 84.64±0.35%, 79.37±0.15%, 70.06±0.13% and 61.32±0.11% at the concentrations of 800µg/ml, 600µg/ml, 400µg/ml and 200µg/ml respectively. Similarly in case of methanolic extract of *M. charantia*, the highest inhibition activity i.e 72.89±0.45% was found at 1000µg/ml followed by 69.72±0.35%, 61.19±1.11%, 37.94±0.86% and 31.67±0.97% at different range of concentration 800µg/ml, 600µg/ml, 400µg/ml and 200µg/ml respectively. The order of percentage of scavenging activity in case of methanolic extract of *E. littorale* was as follows: 70.34±0.26%, 65.37±0.13%, 58.9±0.12%, 52.55±0.17% and 46.65±0.23% at different concentration levels 800µg/ml, 600µg/ml, 400µg/ml and 200µg/ml respectively. (Table-2 and Figure-) The antioxidant capacity is also expressed as 50% inhibitory concentration (IC50). A lower IC50 value means a higher antioxidant capacity of the sample. Significantly lowest IC50 value 142.67µg/ml was observed in methanolic extracts of *Gymnema sylvestre* which is near to 128.35µg/ml obtained in the standard ascorbic acid. The IC_{50} value of the methanolic extract of *E. littorale* leaves and *M. charantia* was 240.02µg/ml and 510.23µg/ml respectively. (Table).

CONCLUSION

In overall comparison the methanolic leaf extract of *Gymnema sylvestre* shows the highest scavenging activity followed by the methanolic extract of M. charantia fruits and methanolic

extracts of e. littorale leaves has been proven as effective antioxidants. It was observed that the antioxidant values were increased with increase in concentration of crude extracts which may be indicated that antioxidant values may be dependent on the presence of different phytochemicals such as phenolics, alkaloids, flavonoids, saponins, tannins etc. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant. The present results revealed that the methanolic extracts of *G. Sylvestre leaves, M. charantia fruits and E. littorale leaves* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the leaves of E. littorale, leaves of *Gymnema sylvestre and Fruits of Momordica charantia are* very much rich in different types of phytochemical constituents especially alkaloids, tannins, saponins, phenols, glycosides, flavonoids etc. So it can be concluded that methanolic extract of *E. littorale* leaves, *G. Sylvestre leaves* and *M. charantia* fruits can be used as an accessible source of natural antioxidant.

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