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Research Article

Pharmacognostical, physiochemical and phytochemical study of herbal plants of an antidiabetic polyherbal formulation

K. Jyothsna Jayaraju[*1] and A. V. Badarinath[2]

[1]*Department of Pharmacy, Research scholar, JNTUA, Anantapuramu, India*
[2]*Professor, Bharat Institute of Technology, Hyderabad, India*

Abstract

Diabetes mellitus illustrates a metabolic disorder of multiple etiologies portrayed by insulin resistance, relative insulin deficiency and hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. Therefore as an alternative of mono-drug therapy the treatment of the disease requires the poly herbal combination therapy. In the present study the investigation carried out on the pharmacognostical, phytochemical and physiochemical features of the different parts of the plants. Polyherbal formulation of extracts of bark of *Cinnamomum zeylenicum*, seed of *Eugenia jambolana*, whole plant of *Vinca rosea*, leaves of *Gymnema sylvestre*. These Plants are fortunate things to us from the nature as they assert their prospective as a wide range of herbs. The comparative morphological studies of different parts showed the presence of various pinpointing characters. The parameters like Ash value, extractive value and moisture content were determined for quality standard of drugs. During the course of the experimental work the different plant parts showed the presence of various phytoconstituents like flavonoids, glycosides, tannins, protein, sterol etc.

Keywords: Phytochemical, Polyherbal, Pharmacognostical, Physiochemical, Extractive value, Moisture content.

***Correspondence Info:**
K. Jyothsna Jayaraju
Department of Pharmacy,
Research scholar, JNTUA,
Anantapuramu, India

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1. Introduction

Diabetes mellitus is one of the most common disorders affecting almost 6% of the world population and the dynamics of the diabetes are changing rapidly in low- to middle-income countries [1]. According to International Diabetes Federation's (IDF) estimates, 80% of the world diabetic population will be from low- and middle-income countries in year 2030. As per IDF 2011 report, China, India, and the US encompass a diabetic population of 90.0, 61.3, and 23.7 million, which may be increased up to 129.7, 101.2, and 29.3 million, respectively, in 2030[2]

Vinca rosea (*Catharanthus roseus*) Linn. Belonging to family Apocynaceae is an herbaceous sub shrub also well-known as Madagascar periwinkle, *Vinca rosea*, or *Lochnera rosea* worldwide. It is cultivated mainly for its alkaloids, which are having anticancer activities [3]. The alkaloids and tannins are the two classes of active compounds in Vinca [4]. The leaves and stems are the sources of dimeric alkaloids, vincristine and vinblastine that are crucial cancer drugs, while roots have antihypertensives like ajmalicine and serpentine [5,6]. The leaves are used traditionally in various regions of the world to manage diabetes [7]. Fresh leaf juice of *C. roseus* has been reported to drop off blood glucose in normal and alloxan diabetic rabbits [8]. Leaves and twigs of plant have been testified to have hypoglycaemic activity in streptozotocin induced diabetic rats [9].

The *E. jambolana* belonging to the family Myrtaceae is familiar as black plumor Jamun, is being widely used to treat diabetes by the traditional practitioners over past decades [10-12]. It is a large evergreen tree growing up to 30m high found widely in India with oval to elliptical fruits are 1.5-3.5cm long, dark purple or nearly black, luscious, fleshy and edible [12]. The extract of *E.*

jambolana pulp illustrated the hypoglycemic activity in streptozotocin induced diabetic mice within 30min of administration while the seed of the same fruit required 24h [13]. The antihyperglycemic activity of *E. jambolana* seeds is well documented [14,15]. It has been reported a decrease in the blood sugar level in alloxan diabetic rats by the intake of the ethanolic extract of *E. jambolana* The flavonoid rich *E. jambolana* seed extract when administered orally to experimental animals in various dose levels were found to be both hypoglycemic and hypolipidemic and shows profound effect on the carbohydrate and lipid metabolism on diabetic rats[16].

Cinnamomum zeylanicum belongs to the Lauraceae family, is the eternal tree of tropical medicine. Cinnamon is one of the most important spices used daily by people all over the world contains cinnamaldehyde, cinnamic acid, cinnamate, etc. It is known to have antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular disease lowering compounds. Cinnamon has also been reported to have activities against neurological disorders, like Parkinson's and Alzheimer's diseases [17]. A substance "insulin-potentiating factor" (IPF) has been isolated from Cinnamon [18] while the antidiabetic actions of cinnamon bark have been expressed in streptozotocin-induced diabetic rats [19]. Numerous studies have also revealed that cinnamon extracts lower not only blood glucose but also cholesterol levels [20–25]. A study comparing the insulin-potentiating effects of many spices revealed that the Cinnamon aqueous extract was 20-fold higher than the other spices [26].

Gymnema sylvestre belonging to family Asclepiadaceae is a slow growing, perennial and medicinal woody climber found in central and peninsular India. Its leaves are called "Gurmar" in India are well known for their sweet taste suppressing activity [27] and are used for the diabetes mellitus treatment [28-31] for over 2000 year, that's why the name "Gurmar" meaning 'sugar destroying'. Gymnema leaf extract administration to a diabetic patient has lead to stimulation of the pancreas by good worth of which there is an increase in insulin release [32]. The effects of an alcoholic extract of *G. sylvestre* on insulin secretion from islets of langerhans and several pancreatic β-cell lines were examined and found to be beneficial [33].

2. Materials and Methods

2.1 Plant material

Leaves of *Vinca rosea*, *Gymnema selvestre*, Bark of *Cinnamomum zeylenicum*, Seeds of *Eugenia jambolana* were collected from local market, Andra Pradesh India. The plant parts were authenticated by Dr. Madhusudhanchetty Head of the Department of Botany, S.V. University, A.P. Then were cleaned and dried under the shade.

2.2 Preparation of plant extract for Phytochemical Screening and Antidiabetic Studies

Leaves of *Vinca rosea*, *Gymnema selvestre*, Bark of *Cinnamomum zeylenicum*, Seeds of *Eugenia jambolana* were shade dried at room temperature and were powdered in a Wiley mill. The extracts were subjected to qualitative test for the identification of various phytochemical constituents.

Cinnamon bark powder approximately 100 g was placed in a Soxhlet apparatus and extracted in aqueous medium for 60 h. The extract was concentrated in a rotary evaporator at reduced pressure to give a yield which was then stored in freezer and used for further studies [34]. The *Vinca rosea* whole plants powder 100 g was used for extraction with 95% methanol in Soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator [35]. *Eugenia jambolana* Seed powder was extracted with Soxhlet using 95% ethanol for 72 hours, concentrated on water bath (70 ^{0}C), kept in oven (30^{0}C) for drying and stored in desiccators [36]. *Gymnema selvestre* leaves powder 100g was extracted with 95% v/v methanol at 60–75^{0}C for 48 hrs. The collected extract was then filtered, concentrated &dried under the reduced pressure by a rotating evaporator and the residue was kept in desicators to remove moisture [37].

2.3 Identification, evaluation and standardization

Identification of herb is based on both the macroscopical and microscopical features. Macroscopical feature comprises odour, taste, color, size, shape and special feature of plant and microscopic features involve leaf content, trichome, stomata etc. Some microscopical features and chemical test comes under evaluation and standardization of herbal drug. Evaluation of drugs connotes confirmation of its identity and determination of its quality and purity and detection of adulteration [38].

Standardization phrase is used to portray all measures which are taken during the manufacturing process and quality control leading to a reproducible quality. It's also entailing the study from birth of plant to its clinical application. In other words it's ensuring that every packet of medicine has accurate ingredient in correct amount and will persuade intended therapeutic effect.

Fig. 1- *Vinca rosea*

Fig. 2-*Cinnamomum zeylenicum*

Fig.3- *Eugenia jambolana*

Fig .4 -*Gymnema selvestre*

2.4 Phytochemical screenings of herbal drugs

Extracts were subjected for Phytochemical screenings to detect the presence phytochemical constituents like alkaloids, carbohydrates, steroids, proteins, tannins, phenolic compounds, flavonoids, gums and mucilage, glycosides by using standard tests[39-42].

2.5 Evaluation Parameters of herbal drugs

Evaluation means authentication of its identity and determination of quality and purity of the herbal drug. Evaluation of crude drug is necessary because of three main reasons that are to detect biochemical variations in the drug, deterioration due to treatment and storage, substitution and adulteration. For the quality control of a traditional medicine, the traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment or monograph in herbal pharmacopoeia [43-45]. The crude drug can be evaluated by following methods:

2.5.1 Macroscopic evaluation

It includes evaluation of drugs by colour, odour, taste, size, shape and special feature, like touch, texture etc. it is the method of qualitative evaluation based on the study of morphological and sensory profile of whole drug [38].

2.5.2 Microscopic Evaluation

It encompasses detailed examination of the drug and it can be employed to identify the organized drugs by their known histological characters. It is mostly used for qualitative assessment of organized crude drugs in entire and powder forms with help of microscope [43,44,46].

2.5.3 Chemical evaluation

Most of drugs have specific chemical constituents to which their biological activity is attributed. Qualitative chemical test include acid value, saponification value, sulphated ash are useful in identification of chemical constituents and detection of adulteration.

2.5.4 Physical evaluation

Physical constants are also taken into consideration to evaluate certain drugs. These include moisture content, optical rotation, specific gravity, refractive, melting point, viscosity and solubility in different solvents. All these physical properties are useful in identification and detection of constituents present in plants.

2.5.4 Physical evaluation

A. Determination of foreign matter [47,48]

It is the foreign matter present in the drug and its presence may be due to faulty collection of crude drug or due to intentional mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated. Drugs should be free from insects, moulds, animal, faecal matter and other contamination.

Percentage of foreign organic matter =

$$\mathbf{n \times W \times 94{,}100 \times 100 / S \times M \times P}$$

Where,

n= number of chart particles in 25 fields.

S= number of spores in the same 25 field.

W= weight in mg of lycopodium taken.

M= weight in mg of the sample (calculation on the sample dried at 105^0C)

P= number of characteristics particles per mg of the pure foreign matter.

94,000= number of spores per mg of lycopodium.

B. Determination of total ash

The remains after incineration is the ash content of drugs, which simply represents inorganic salts which are naturally occurring in drugs or adhering added to it as form adulteration[49].

Two types ash determine-

(i) Acid insoluble ash value.

(ii) Determination of water soluble ash.

C. Determination of extractive value

(i) Determination of alcohol soluble extractive

(ii) Determination of water soluble extractive

D. Determination of moisture content

Weigh 10 gm of drug and is taken in an evaporating dish. Then it is dried at 105°C for 3 hours and again weighed. Drying and weighing was carried on at one hour interval until difference of two successive weighing matches to not more than 0.25 percent. The reading is taken after a constant weight is attained and the moisture content is determined [50].

E. Determination of pH

The pH value of an aqueous liquid may be defined as the common-logarithm of the hydrogen ion concentration expressed in grams. Potentiometrically pH value is determined by a glass electrode and a suitable pH meter [38].

F. Solubility

The presence of adulterants in a drug could be indicated by solubility studies identify by various solvents [38].

I. Alcohol

5 gm of powdered drug material along with 100 ml of alcohol are shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquefied extract obtained was concentrated in a vacuum oven and the percentage was calculated with the weight of the drug powder taken.

ii. Water

The procedure adopted for solubility percentage of alcohol is used with chloroform water instead of alcohol to get the water solubility.

G. Refractive index

When a ray passes from one medium to another of different density, it is bent from original path. Thus, the ratio of velocity of light in vacuum to its velocity in a substance is termed as refractive index of the second medium.

H. Swelling Index

5g of plant material previously reduced to the required fineness and accurately weighed taken into 25 ml glass stopped measuring cylinder. 25ml of water was added and the mixture was shaken thoroughly every 10 min for 1 h. It was allowed to stand for 3 hrs at room temperature. The mean value of the individual determinations was calculated related to 1g of plant material.

I. Foaming Index

3 g of plant material was reduced to a coarse powder, weighed accurately and transferred at moderate boiling for 30 min. Cooled and filtered into 100 ml volumetric flask. The decoction was poured into 10 ml flask and adjusted the volume of liquid in each tube with water to 10 ml. The tubes were closed and shaken in a lengthwise motion for 15 sec.; two shakes per second. Allowed to stand for 15 min and the height of foam were measured.

3. Results

Table 1: Comparatively Moisture content (%w/w), Ash value (%w/w) and Swelling index of *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre*

S. No.	Particular	Moisture content(%w/w)	Total ash	Acid insoluble Ash	Water soluble Ash	Swelling index(in ml)
1	***Cinnamomum zeylenicum***	3.3±0.14	5.26%	0.59%±0.04	3.08%±0.04	4.65±0.05
2	***Vinca rosea***	2.9±0.23	4.2%	0.61%±0.13	2.1%±0.11	3.20 ±0.45
3	***Eugenia jambolana***	3.4±0.44	6.5%	0.41%±0.07	4.0%±0.21	3.40 ±0.32
4	***Gymnema selvestre***	3.6±0.05	5.8%	0.23%±0.17	3.1%±0.07	4.15±0.08

All values are Mean (n) ± SD, n=4 and SD= Standard Deviation

Table 2: Comparatively Qualitative phytochemical screening of *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre*

Chemical constituents	*Cinnamomum zeylenicum*	*Vinca rosea*	*Eugenia jambolana*	*Gymnema selvestre*
Alkaloids	++	++	+	+
Saponins	+	+	+	++
Tannins	++	+	++	+
Phenolic compounds	+	+	++	+
Flavonoids	++	+	++	+
Terpenoids	++	+	+	+
Steroids	++	+	+	+
Glycosides	+	+	+	+
Proteins and amino acids	+	+	+	+
Carbohydrates	+	+		+
Oils and fats	+	_	_	_

++= presence in more amount, += Presence, - = Absence

Table 3: Comparatively Morphological characters of *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre*

Parameters	*Cinnamomum zeylenicum bark*	*Vinca rosea whole plant*	*Eugenia jambolana seeds*	*Gymnema selvestre leaves*
Color	Dull yellowish brown	Flowers are pink and white, leaves stems are green	Dark Cream	Green
Odour	Pleasant	Characteristic	Characteristic	Characteristic
Taste	Sweet	Characteristic	Pungent and sour	Tasteless
Size and shape	15-25cm long, 0.1 cm thick	Erected procumbent herb, leaves are oval to o blong, 2.5 – 9.0cm long. Flowers are 2.5 – 3.0 cm long with five petals like lobes.	1-2 cm long,	2-4cm long, and ovate shape

Table 4: Comparatively Extractive values (%w/w), foreign matter content (%w/w) and Foaming index of *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre*

S. No	Particular	Alcohol (%w/w)	Water (%w/w)	Foreign matter	Foaming index
1	*Cinnamomum zeylenicum*	54±1.7	32±0.4	1.2±0.2	<100
2	*Vinca rosea*	50 ±1.4	35±0.42	1.6±0.9	<100
3	*Eugenia jambolana*	47±0.11	41±1.2	0.7±1.3	<100
4	*Gymnema selvestre*	55±1.04	45±0.8	1.03±0.8	<100

All values are Mean (n) ± SD, n=4 and SD= Standard Deviation.

4. Discussion

Standardization of *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre* was according to WHO guidelines. The results obtained found were under specified limits.

Morphological study is a prominent part for the identification of any drug. Morphological study illustrated that the drugs *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre* are having the characteristics as per the standards given in official compendia. Foreign matter is the material containing of any organ other than those named in the identification and description, matter not coming from the source plant, molds, insects or other animal contamination. The results illustrated the content of foreign matter is 1.8 ±0.13, 0.8 ±0.14 and 1.02±0.16 respectively present in the drugs.

Moisture content is used to determine the presence of volatile matter (i.e. water drying off from drug). The moisture content is 3.3±0.14, 2.9±0.23 and 3.4±0.44 respectively maximum moisture content.

The total ash value which show the presence inorganic matters in drug were (5.26%, 4.2%, 6.5%, 5.8% respectively), acid insoluble ash (0.59%±0.04, 0.61% ±0.13 and 0.41% ±0.07, 0.23% respectively) and water soluble ash (3.08%±0.04, 2.1% ±0.11, 4.0±0.11% and 3.1 ±0.21 respectively) was found in drugs.

Foaming index was found in less than 100 in *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana* indicating absence of saponins but more than 100 in *Gymnema selvestre indicating* presence of saponins. No Swelling was found to be 4.65±0.05, 3.20 ±0.45 and 3.40±0.32 respectively. It showed that polar constituents present in large quantity than non-polar constituents in the drugs.

The extractive value is used to specify miscibility and presence of constituents in particular solvents, it is performed in solvents like alcohol and water as they indicate presence of more polar compound on moving on polarity scale.

The extractive value in alcohol was found to be 54±1.7, 50±1.4, 47±0.11 and 53%±1.04 w/w respectively. The extractive value in water was found to be 32±0.4, 35±0.42, 41%±1.2, 45%±0.8w/w respectively by Soxhlet method.

5. Conclusion

In conclusion, the present study has shown that the investigation of polyherbal formulation of extracts of *Cinnamomum zeylenicum, Vinca rosea, Eugenia jambolana, Gymnema selvestre was* carried out to certain findings about the pharmacognostical, physiochemical and phytochemical aspects which no uncertainty can be proved valuable and provide as scientific background for auxiliary isolationary steps to obtain the lead.

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