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Pharmacognostic and Preliminary Phytochemical Investigation of *Gynandropsis gynandra* Linn.

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ABSTRACT

*Gynandropsis gynandra*Linn is an erect, hairy herbaceous annual herb belonging to Capparaceae family. It is used for its dietary and antioxidant characteristics in different traditional systems. In India alone, it is used in for many illnesses such as epilepsy, irritable bowel syndrome, in protozoa and worm infections.The study determines different pharmacognostical and phytochemical standards that are helpful in ensuring the purity, safety and effectiveness of *Gynandropsis gynandra*Linn. Whole leaves, powdered leaves and extracts were studied macro and microscopically and standardization parameters have beenestablished in accordance with WHO guidelines. Parameters include extractive values, ash values and loss on drying. Preliminary phytochemical screening of extracts was also performed. For intact drug and powdered drug material of *G. Gynandra*, the shape, size, color, odor and surface features were noted. Microscope picture of cross section of the leaf and powdered microscopy showedimportant diagnostic characteristics. Phytochemical and physicochemical study established useful tools to distinguish the powdered drug material. β-carotene was quantified by HPTLC. The information formed from thisresearch will assist to validate the medicinal importance of*G. gynandra*. Qualitative and quantitative microscopic characteristics may be useful for setting the norms as per pharmacopeia. Morphology as well as different pharmacognostic features of distinct components of the plant were studied and explained along with phytochemical and physicochemical parameters that could be useful in further isolation and purification of medicinally significant compounds.

KEYWORDS

Pharmacognostic, Phytochemical, Gynandropsis gynandra.

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INTRODUCTION

*Gynandropsis gynandra*Linn.(syn. *Cleome gynandra*Linn, *C. Pentaphylla* Linn.) is an erect, hairy herbaceous annual herb belonging to Capparaceae family. It is never grown in India, but developed in all places spontaneously. Different Gynandropsisspecies can be discovered in all Indian states. It is used for its dietary and antioxidant characteristics in different traditional systems. In India alone, it is used in for many illnesses such as epilepsy, irritable bowel syndrome, in protozoa and worm infections[4]. It is a chief constituent of Ayurvedic medicine named Narayana Churna[1,2].

Phytochemical study on leaves of *Gynandropsis gynandra* has shown that the leaves contain 5% protein, 6% carbohydrates and are high in vitamins A and C, calcium, phosphorus and iron. The bitter flavorcomes from polyphenolics, which make up from 0.5% to 0.9% of the edible leaf[3]. A decoction of cooked leaves was used to treat stomach-ache, constipation, conjunctivitis, thread-worm infection, chest pain and to facilitate childbirth in pregnant women[3,4]. The standardization process can be accomplished through stepwise Pharmacognostical research. In current study, different pharmacognostical and phytochemical standards of *Gynandropsis gynandra*leaves have been evaluated.

MATERIALS AND METHODS

Plant collection and Identification

The plants of *Gynandropsis gynandra*Linn.(Capparaceae) were collected from roadside area of Manjalpur, Vadodara, Gujarat, India. Plant was authenticated at Botanical Survey of India, Pune.

Pharmacognostical studies:

Macroscopic and microscopic investigation

The macroscopic features of leaf of *Gynandropsis gynandra*Linn. was determined using prescribed method[5]. For microscopical examinations, the transverse sections of leaf were obtained by using rotary microtome.The powder microscopy was conducted according to prescribed technique[5,6].

Leaf constant

Leaf constants such as stomatal index, vein islet number, vein termination number and palisade ratio were studied by conventional techniques[5,6].

Physicochemical analysis:

Plant leaves powder was evaluated for various Physicochemical analysis like extractive value, Loss on drying, Ash

values (Total, Acid-insoluble and Water soluble ash)[5,7].

Elemental Analysis

Elemental analysis of leaf of *G. gynandra* was performed to determine the presence of heavy metals and other inorganic elements[5].

Preliminary phytochemical screening

Shade dried leaves powder has been extracted successively with petroleum ether, chloroform, ethyl acetate, methanol and water. The extracts were filtered and concentrated using vacuum distillation. The extracts have been subjected to qualitative tests to identifydistinct phytoconstituents as per standard procedure[5,8,9].

HPTLC

HPTLC study was done to quantify β-carotene in PE:Acetone extract[8,10].

Standard Preparation

Stock solution of standard β carotene was prepared by dissolving 10mg in mixture of PE:Acetone (1:1). From this stock solution dilutions were prepared to get concentration range from 200-600ng.

Sample Preparation

Leaves of *G.gynandra* were extracted with petroleum ether: acetone (1:1) overnight. It was filtered and filtrate was washed with water. The water layer was discarded. Organic layer was concentrated and was used for sample application on pre-coated silica gel 60F254aluminium sheets.

Developing Solvent System: Several solvent systems have beentested, for extracts, but the adequate resolution was obtained in the solventn-Hexane: Benzene (9:1).

Sample Application: Standard and extract bandswere applied using spray technique. They were applied on pre-coated silica gel 60F254 Aluminum sheets (10 x 10 cm) with the help of Linomat 5 applicatorattached to CAMAGHPTLC system, which was programmed through WIN CATSsoftware.

Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber saturated with solvent n-Hexane: Benzene (9:1) for 25 minutes. The air-dried plates were observed.Thechromatograms were scanned by densitometer at 445 nm. The R_f values and finger printdata were recorded by WIN CATS software.

RESULTS AND DISCUSSION

Pharmacognostic study:

The thorough and systematic pharmacognostical evaluation would offersignificantdata for the future studies.

Macroscopic study:

The plant leaves contain up to 7 leaflets spreading like the fingers of the palm and leaflets growing up to 8 cm long. Leaves are

alternate, palmately compound with 3 to 7 leaflets, Elliptic obovate in shape with entire margin,cuneate base, palmately reticulate venation and pubescent surface.

Figure 1a *G. gynandra* Plant

Figure 1b *G. gynandra* Leaf

Microscopic study:

It is a dorsiventral leaf.

The upper and lower epidermis is made up of single layer of polygonal cells; cells have wavy cuticle walls. Both covering and glandular trichomes come out from the epidermal layer. Covering trichomes are uniseriate, multi-cellular and pointed at the apex. Glandular trichomes consist of multicllular stalk and multicellular glandular head.

Mesophyll is differentiated in to palisade and spongy parenchymatous cells. Palisade cells are single layered, loosely and irregularly arranged and does not form a continuous band throughout as it is absent above the vascular bundles of midrib. Spongy parenchyma 2-3 layered with large intercellular spaces. Fibrovascular bundles are seen in lamina of *G.gynandra* leaf.

The collateral vascular bundle occupies the midrib's central part. Xylem is on the ventral surface and phloem is on the dorsal surface. A separate endodermal layer surrounds vascular bundle.

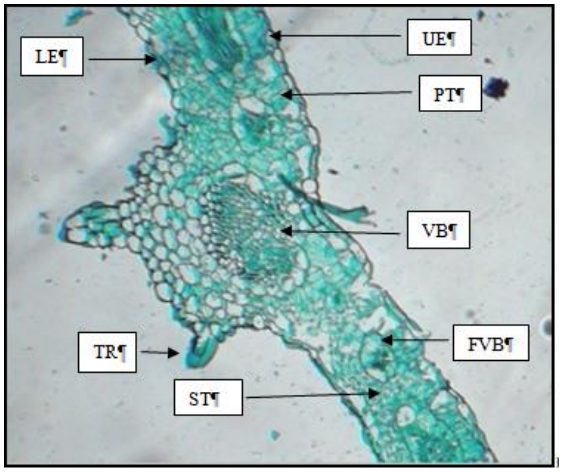

Figure 2 Transverse section of leaf of *Gynandropsis gynandra*

UE: Upper epidermis; PT: Palisade tissue;ST: Spongy tissue; FVB: Fibrovascular bundle; VB: Vascular bundle; LE: Lower epidermis; TR: Trichome

Powder study of the *G. gynandra* leaf powder

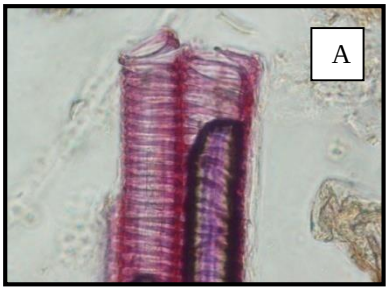

Figure 3 A. Xylem vessels; (one

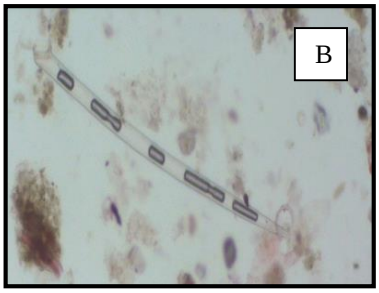

Figure 3 B. Trichome;

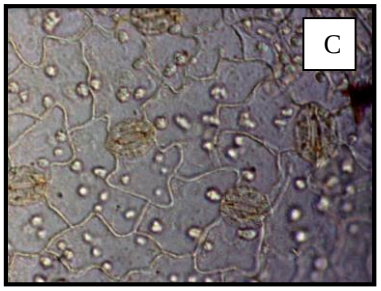

Figure 3 C. Anisocytic stomata

heading for three figures)

The results of Leaf constants such as stomatal number, stomatal index, vein-islet number and veinlet terminations number were shown in Table 1.

Table 1 Leaf constant of leaves of *G. Gynandra*

Sr. No.	Parameter	Values (in 1mm^2 area)
1	Stomatal index	17.5 -18.00
2	Stomatal number	27.00 - 27.34
3	Vein islet number	9.5
4	Vein termination number	6.4
5	Palisade ratio	10.4

Studies on physico-chemical constants can be serving as an important basis of information and give suitable standards to establish the quality of this plant. In the current study, physical constant as drug's total ash value shows contamination with foreign matter such as metallic salts or silica and other impurities present along with drug. The quantity of acid-insoluble ash shows contamination with earthy and siliceous material and the water soluble ash is used to identifywater exhausted materials. Extractive values are helpfulfor evaluating the chemical components present in the crude drug as well as helpingto estimate specific components soluble in particular solvents. The outcomes of the physical constants of the drug powder are shown in table-2.

Table 2 Physico-chemical analysis of leaves of *G. Gynandra*

Sr. No.	Parameter	Average values %w/w
1	Extractive value	
	Water soluble	5.25
	Alcohol soluble	2.0
2	L.O.D	10.0
3	Ash value (Total)	21.25
	Water soluble	8.5
	Acid insoluble	2.0

Contamination of the medicinal plant materials with heavy metals can cause chronic or acute poisoning. Therefore it has become necessary that all the starting materials should be ensured for their heavy metal content including other necessary inorganic elements.Heavy metal content and other inorganic elements of *G. gynandra* leaves are mentioned in table 3.

Table 3 Content of heavy metals and other inorganic elements of leaves of *G. Gynandra*

Sr. No.	Parameter	Content present (ppm)
1	Manganese	47.0
2	Zinc	100.0
3	Copper	17.62
4	Sodium	2836.0
5	Iron	0.14%

6	Cadmium	1.37

The preliminary phytoprofile of extracts obtained by successive solvent extraction isgiven in table 4. The values depend on the chemical nature, quality, properties of constituents, the solvent employed, and the type of plant part and the method of extraction employed.

Table 4 Preliminary phytoprofile for leaves of *G. gynandra*

Sr. no.	Solvent	Color	Consistency	%Yield w/w
1	Petroleum Ether	Yellowish green	Sticky	2.86
2	Chloroform	Blackish green	Sticky	1.63
3	Ethyl Acetate	Blackish green	Sticky	1.16
4	Methanol	Green	Sticky	5.2
5	Water	Brown	Dry non sticky	8.13

In order to determine the existence of secondary plant metabolites, the extracts extracted from the consecutive solvent extraction method were subjected to multiple qualitative chemical tests.The results of preliminary phytochemical screening of extracts of *G. gynandra* leaves are shown in table 5.

Table 5 Qualitative chemical test of different extracts of aerial part of *G. gynandra*

Sr. No.	Chemical constituent	P.E extract	$CHCl_3$ extract	E.A. extract	MeOH extract	Water extract
1	Carbohydrate	-	-	-	+	+
2	Protein	-	-	-	-	-
3	Amino Acid	-	-	-	-	-
4	Saponins	-	-	-	+	+
5	Steroids	+	+	-	-	-
6	Alkaloids	-	-	-	-	-
7	Terpenes	+	+	-	+	-
8	Tannins & phenolics	-	-	-	+	+

P.E – Petroleum ether,$CHCl_3$ – Chloroform**,** E.A – Ethyl acetate, MeOH – Methanol
+ Positive - Negative

HPTLC:

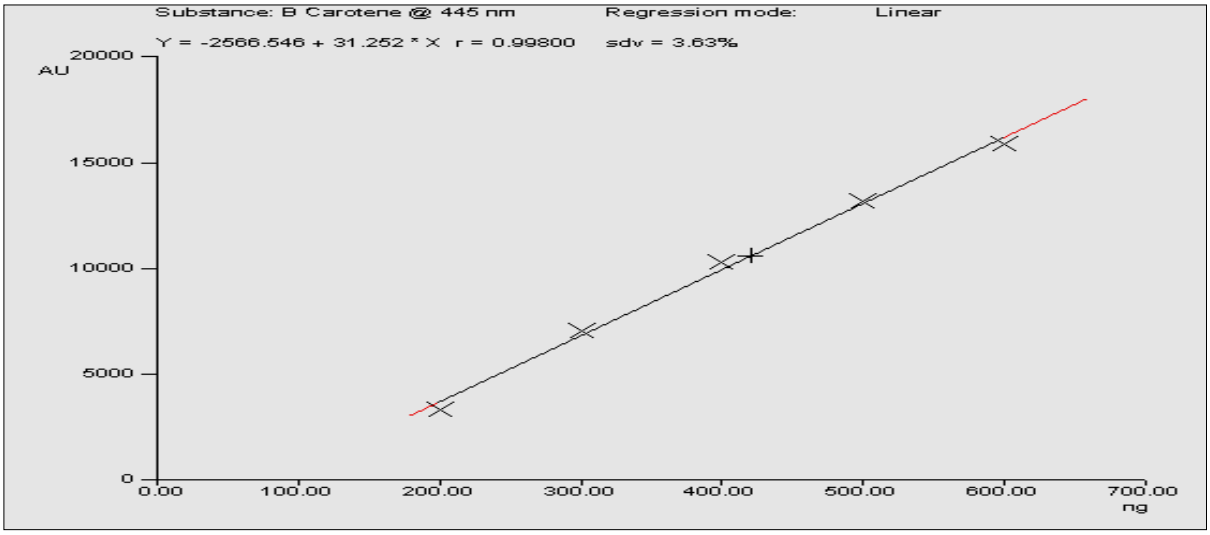

Figure 4 Calibration curve of standard β-carotene

The percentage content of β-carotene in PE:Acetone extract of *G.gynandra* leaves extract was found to be 1.8% w/w. This was determined by a calibration curve with the equation of Y= -2566.546+31.252*X (correlation coefficient = 0.9980 and standard deviation = ± 3.63%) as shown in Figure 4, where X represents the amount of β-carotene and Y represents area under the curve.

R_f value of standard β-carotene was found to be 0.53[Figures 5-8].

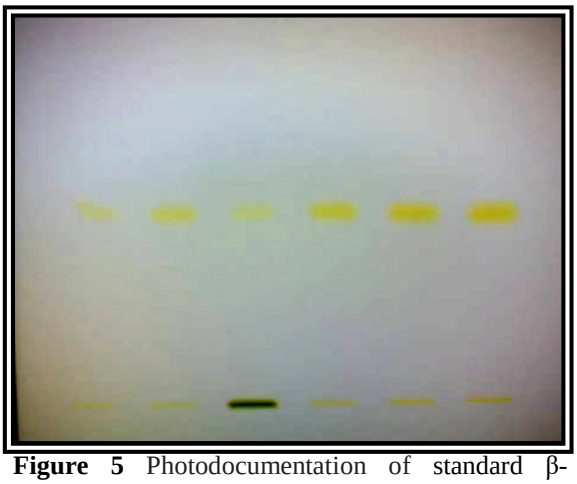

Figure 5 Photodocumentation of standard β-carotene and PE:Acetone extract of *G.gynandra* leaves at 445 nm

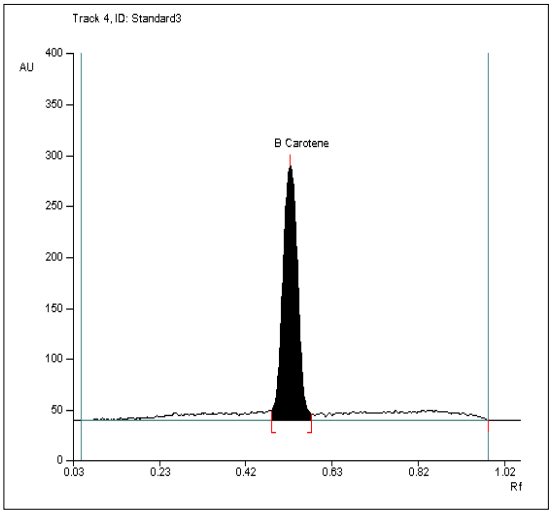

Figure 6 HPTLC chromatogram of standard β-carotene

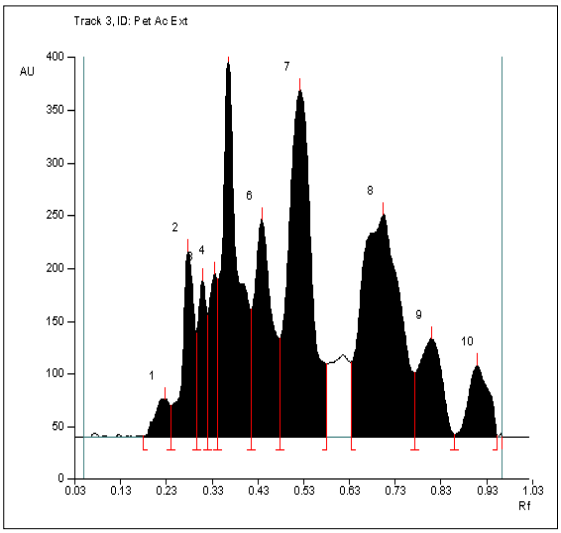

Figure 7 HPTLC chromatogram of PE:Acetone extract of *G.gynandra* leaves

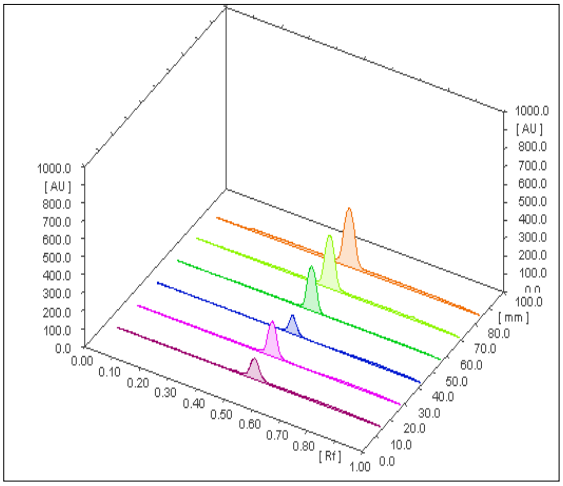

Figure 8 3D chromatogram of standard β-carotene and PE:Acetone extract of *G.gynandra* leavesat 445 nm

CONCLUSION

As a basis for adequate plant identification and investigation, pharmacognostic research and phytochemical screening can be used.These parameters are to be helpful in the herbal monograph preparation for its proper identification. Since *G. gynandra* is used in various medicinal preparations; hence the present study may be useful in the development of pharmacopoeial standards

for the *Gynandropsis gynandra*Linn. in the future studies.

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