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Research Article

Pharmacognostic and Phytochemical Studies on Roots of *Agave Americana* (Agavaceae)

*Kadam P. V, Yadav K. N, Deoda R.S, Narappanawar N. S, Shivatare R. S, Patil M. J

Marathwada Mitra Mandal's, College of Pharmacy, Thergaon (Kalewadi), Pune-411033

ABSTRACT

Now-a-Days medicinal plants found on earth have renowned medicinal significance and their usages are increasing day by day in our daily life. Different researches are going on to explore the beneficial, pharmacological and medicinal properties of herbal drugs. Sophisticated modern research tools for evaluation of the plant drugs are available today but microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. *Agave americana* L commonly known as Century plant has been described as a useful remedy for various medicinal uses, but the pharmacognostic and phytochemical standardization of the roots were not validated till date. The present study deals with pharmacognostical parameters for the root of *Agave americana* L which mainly consists of macromorphology, microscopical characters, physio-chemical constants and phytochemical screening. This information will be of used for further pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity and sample identification.

Key words: *A. americana*, Agavaceae, Flavonoids , Glycosides, Roots, Standardization.

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani [1] because the medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases [2]. Today, we are witnessing a great deal of public interest in the use of herbal remedies [3]. Medicinal plant are moving from fringe to main stream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals [4]. Generally herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [5]. World Health Organization currently encourages, recommends and promotes traditional herbal remedies as such drugs are easily available in low cost, are comparatively safe and the people have faith in such remedies [6]. It is no wonder that the world's one-fourth population i.e. 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments [7]. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine [2]. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. *Agave americana* (Synonyms: American aloe, Rakaspattah, Jangli ananas, Ghaypat) belonging to the family Agavaceae commonly known as Century plant which is widely distributed in in Europe, South Africa, India, and Australia. It has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings. Traditionally, its roots have Diuretic and antisyphilitic properties. Sap is laxative, diuretic and emmenagogue, it is found useful in scurvy, the juice which yields on cutting the leaves and the roots are especially useful in syphilis [8,9,10]**.** Hence, in this work we report some pharmacognostical, physicochemical and phytochemical characteristics. The main objective of this study is to supplement some information with regards to its identification, Characterization and standardization of *Agave americana* root.

MATERIAL AND METHODS

Plant collection, identification and processing: The fresh bark of *Agave americana* was collected in the month of November from Pirungut, Pune District, Maharashtra state, India. The plant was identified and authenticated by Botanical Survey of India, Pune and a voucher specimen was deposited with a voucher specimen sample No. AGANUN2. The fresh roots were firstly dried in shade for 20 days. The fresh root was used for the study of macromorphological and microscopical characters; whereas the dried root powder was used for determination of powder microscopy, physicochemical characterization and phytochemical analysis [11].

Macrmorphologcal Description: The root was subjected to macroscopic studies which comprised of Organoleptic

Author for correspondence: Email: prasadvkadam@indiatimes.com

Fig No.1 Morphological features of *Agave* root

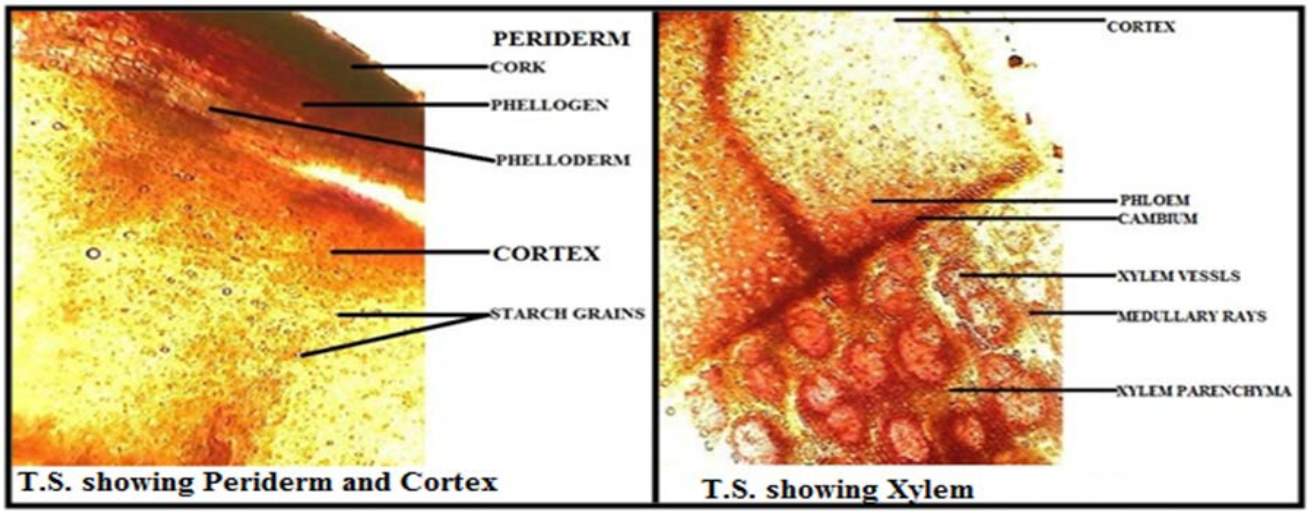

Figure 2: T.S of *A.americana*

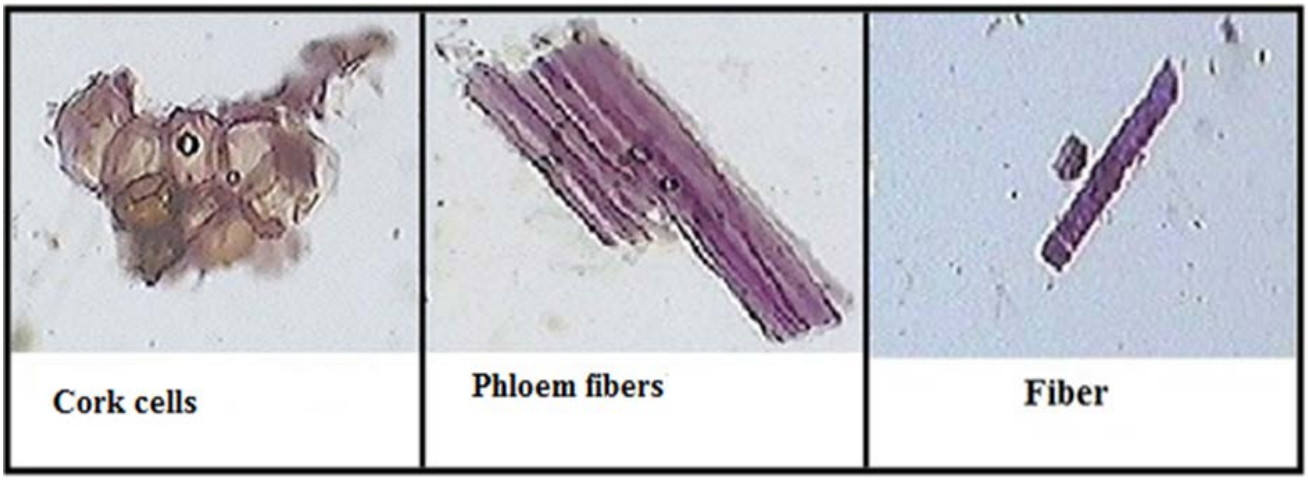

Figure 3: Powder Characteristics of *A.americana* (root)

characteristics viz. color, odour, appearance, taste, shape, fracture, etc. of the drug. These parameters are considered as quite useful in quality control of the crude drug and were evaluated as per standard WHO guidelines [12,13,14].

Microscopic characteristics: Free hand transverse sections (T.S.) of fresh *Agave americana* roots were taken and stained with different but specific staining reagents. Histochemical reactions were applied with staining reagents on transverse sections and on root powder by reported methods. Photomicrographs of the microscopical sections were taken with the help of MOTIC Digital Microscope, provided with MOTIC IMAGE PLUS 2.0 software [12,13,14].

Powder characteristics: Powder microscopy has been done along with use of different microchemical reagents as per standard procedures mentioned [15,16].

Physicochemical evaluation: The dried plant material was subjected for determination of physicochemical parameters such as foreign organic matter, all types of Ash Values, alcohol soluble extractive and water soluble extractive, moisture content and Foaming index. Analysis of Physicochemical Constants of the ingredient has been

Table 1. Macroscopic characters of roots of *A. americana*

Characters	Observation
Colour	Brown
Odour	Characteristic
Taste	Bitter
Surface	Rough and hard
Fracture	Short, irregular

Table 2. Physicochemical parameters of root of *A.americana*

Parameters	Values obtained
Foreign matter (%w/w)	0.5
Total ash (%w/w)	12.16
Acid insoluble ash (%w/w)	1.6
Water soluble ash (%w/w)	6.8
Water soluble Extractive (%w/w)	6.8
Alcohol soluble Extractive (%w/w)	13.8
Moisture content (%w/w)	9.42
P^H (1% solution)	3-4
P^H (10 % solution)	3-4
Foaming index	102.03

Table 3. Fluorescence analysis of root of *A.americana*

Solvents	Visible light	Short UV (254 nm)	Long UV (366 nm)
Drug powder as such	Light brown	Light brown	Light brown
Drug + Conc. H_2SO_4	Brown	Greenish yellow Fluorescence	Light brown
Drug + Conc. H_2SO_4 + Distilled water	Light brown	Brown	Brown
Drug + Conc. HCl	Light brown	Black	Brown
Drug + Conc. HCl + Distilled water	Light brown	Greenish yellow Fluorescence	Brown
Drug + Conc. HNO_3	Muddy brown	Light brown	Dark brown
Drug + Conc. HNO_3 + Distilled water	Light brown	Greenish yellow Fluorescence	Brown
Drug + Methanol	Colourless	Colourless	Colourless
Drug + Chloroform	Light brown	Greenish yellow Fluorescence	Black
Drug + Pet. Ether			
Drug + Ferric chloride (10%)	Dark brown	Greenish yellow Fluorescence	Black
Drug + Picric acid	Yellow	Light brown	Black
Drug + 10% Sodium hydroxide	Brown	Brown	Black
Drug + Ammonia solution	Dark brown	Black	Black
Drug + Distilled water	Light brown	Greenish yellow Fluorescence	Light brown

done to evaluate the quality and purity of the powder drug. Determination of these physicochemical constants was done as per procedures mentioned in accordance with WHO guidelines [11,17,18].

Fluorescence analysis: The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (254 nm and 366 nm) and after treatment with different reagents like sodium hydroxide, hydrochloric acid, nitric acid and ferric chloride etc [19,20].

Preliminary Phytochemical screening: The powder of roots of *A.americana* was subjected to successive extraction using soxhlet apparatus. The solvent according to the polarity index were been selected viz. Petroleum ether (60-80), Ethanol and Water. Preliminary qualitative phytochemical analysis of this extract was carried out by employing standard conventional protocols [13].

Table 4. Phytochemical screening of root of *A. americana*

Tests	Pet ether extract	Ethanolic extract	Aqueous extract
Carbohydrates	+	+	-
Reducing sugar	+	+	-
Alkaloids	-	-	-
Volatile oil	-	-	-
Tannins	-	-	-
Steroids	+	+	-
Cardiac glycosides	+	-	-
Saponin glycosides	-	+	+
Flavonoides	-	-	-

+ indicates presence

RESULTS AND DISCUSSION

Macroscopic analysis: *Agave americana* is relatively fast growing, to about 6 feet tall by 8-10 feet wide. The wide, grey leaves have stiff terminal spines and recurved teeth along the margins. Century plant prefers full sun exposures and well drained soils, but is adaptable to a wide range of conditions, including coastal climates. The organoleptic evaluation of the root showed brown colour with its taste bitter and characteristic odour. The results are showed in Table no. 1. Fig 1. Describe the general morphological appearance of *Agave* root.

Microscopic characteristics: The microscopic characters when observed revealed the presence of typical root characteristics as Periderm which consists of cork, phellogen and phelloderm. Cork was observed dark brown in colour. It is composed of multiple layers, with narrow and tangentially elaongated cells. Phellogen and Phelloderm were observed one beneath the other and are compactly arranged layers of cells. These are 2-3 layered cells which are yellow in colour. The periderm then originates in cortex region. The cortex region is found just below the periderm layer. These are thin walled parenchynatous cells. It showed presence of starch grains in the parenchymatous cells (Fig 2). Phloem is observed just above the cambium. The Secondary xylem showed presence of lignified xylem vessels with simple or bordered piths. The medullary rays run radially from the xylem up to one to five cells in width. Xylem parenchyma is moderately thick walled, lignified with pitted walls.

Powder Microscopy*:* Powder bark is brown, non aromatic, bitter. The microscopic examination of the powder shows fragments of Parenchymatous cells, medullary rays and fibers (Fig. 3).

Physicochemical analysis: Table 2 mentions the results of the physicochemical constants of raw material which lie within the limit; this signifies that the quality and purity of raw material was good enough; the results of foreign organic matter denote presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned which was found to be 0.5 ± 0.28, it indicates that their may be present of part or product of an organism in significantly less amount. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of crude drug affects "total ash" values, such variables are then removed by treating with acid (as they are soluble in hydrochloric acid) and then acid-insoluble ash value is determined. The result of total ash value indicated the purity of drug that is the presence or absence of foreign matter such as metallic salt or silica present in the crude drug; the values were found to be 12.16%w/w [19]**.** Acid insoluble ash particularly indicates contamination with silicious materials e.g., earth and sand, comparisons of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug which was found to be 1.6%w/w. The water soluble ash was found to be 6.8%w/w; this parameter is used to detect the presence of material exhausted by water whereas the value for Ash the ash values of the crude drugs lies with in the fair limit which signify its quality and purity and gives idea about the total inorganic content. Extractive values are used to determine the amount of active constituents in given amount of medicinal plants which is qualitative as well as quantitative estimation of phytoconstituents which act as a preliminary information about the drug; the water soluble extractive value found to be 6.8%w/w. While the alcohol soluble extractive value was found to be 13.8%w/w which signify the nature of the phytoconstituents present in plant. Deterioration time of the drug depends upon the amount of water present in formulation. If the water content is high, the formulation can be easily deteriorated due to fungus and the moisture content of the drug was found to be 9.42%w/w which signify that the drug was properly dried and properly stored. The pH was in the range of 3 to 4 which was in acidic range and may be because of acidic salts present in the drug. Foaming index is a significant characteristics for the presence of saponins in the crude drug extract. It was found to be 102.03 which signifies the presence of saponin like glycosides as indicated in Table 2

Fluorescence analysis: The results of fluorescence analysis were expressed in Table 4. Fluorescence study is an essential parameter for first line standardization of crude drug. In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight. (Table 3)

Phytochemical screening: The preliminary phytochemical investigations of powdered Roots were performed which

shows the presence secondary metabolites which is summarized in the Table no.4

CONCLUSION

Standardization is essential measure for quality, purity and sample identification. The present study on Pharmacognostical & Phytochemical evaluation of roots of *Agave americana Linn* family Agavaceae will provide useful information for its identification. *Agave americana roots* have, flavonoids, and saponins which have therapeutic value. Generated data can be used for determining correct identity and purity of plants part and detection of adulteration as well. Hence, detailed screening may be done to isolate the active constituent so that it may be scientifically proved to access the pharmacological responses of the plant to ascertain it's folklore uses.

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