ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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Pharmocognostical and Phytochemical Evaluation of Stem of *Artabotrys odoratissimus.(roxb.)R.Br.(Annonaceae)*

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Article History:

Received on: 27 Jun 2020
Revised on: 25 Jul 2020
Accepted on: 03 Aug 2020

Keywords:

Extraction, organoleptic, microscopy, physico-chemical, Mineral Analysis

ABSTRACT

Medicinal plants constitute the major bioactive compounds of most indigenous medicines and a large number of medicinal preparations which contain one or more ingredients of plant origin. The worldwide enthusiasm for the study and practice on herbs has in this manner, significantly expanded during the most recent two decades due to developing mindfulness about the quality and symptoms of manufactured medications, their impediments in numerous zones of treatment, nearly significant expense and regularly tranquilize past the range of normal man. For thousands of years people have utilized herbs for health care. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Pharmacognostical studies help in the identification and authentication of the plant compounds isolated from various parts of plants. Stem bark of *Artabotrys odoratissimus* were studied by macroscopical, microscopical, physicochemical, phytochemical, fluorescence analysis of powder and other methods for standardization recommended by WHO. The pharmacognostic characters investigated, will help in identification of the crude drug; the standardization parameters set down will guarantee the viability of medication and furthermore recognize the medication from its adulterants. The particular characters will moreover be valuable for the arrangement of monograph of this plant.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i1.4185

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INTRODUCTION

Medicinal plants are playing most active role in traditional medicines for the treatment of various diseases (Chandra *et al.*, 2007; Md *et al.*, 2009). However, a key obstacle, which has hindered the promotion in use of alternative medicines in the developed countries, is no evidence of documentation and absence of stringent quality control measures (Siddiqui and Husain, 1994; Jain *et al.*, 2006).

There is a need for the record of all the research work carried out on traditional medicines in the form of documentation. With this drawback, it becomes extremely important to make surety about the standardization of the plant and parts of plant to be used as a medicine. For the process of standardization, we can use different techniques and methodology to achieve our goal in the stepwise manner *e.g.* pharmacognostic and phytochemical studies (Saxena and Tripathi, 1989; Qureshi and Bhatti, 2004).

These steps and processes are helpful in identification and standardization of the plant material (Md *et al.*, 2011). Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy (Ahmad *et al.*, 2006; Willow, 2011). For this purpose we have done pharmacognostic studies of *Artabotrys odoratissimus* (*Annonaceae*) (Benzie and Wachtel-Galor, 2011; Odugbemi, 2008). *Artabotrys odoratissimus* R.Br. (Table 1) is a shrub native to eastern Asia and belongs to Annonaceae family (Hasan *et al.*, 1987).

This plant is commonly known as Manoranjini (Malayalam), Manoranjitham (kannada), Harchampa (Hindi), Kalchampa (Beng), Lilochampa (Guj), and Harachampaka (San). It is a straggling shrub, leaves oblong - lanceolate, 6-10 x 2-4 cm long, acuminate, cuneate at base, shining above. Flowers fragrant, solitary or few flowered, peduncle. Sepals 3, recurved. Petals 6 in two whorls of 3 each, clawed at base, green at first turning yellow. Berries yellow 6-10 in a cluster. Flowering and fruiting occur most part of the year. It is indigenous to Indian Peninsula and Srilanka (Sastri, 1950)

Medicinal properties and uses

Artabotrys odoratissimus (Figure 1) is an ornamental plant (Mehta *et al.*, 2002). Fruits of this plant are recorded as containing fixed and volatile oil glycosides and resins, extracts are reported to exhibit hypotensive and spasmogenic as well as cardiac stimulating effects on some animals and cardiac depressant on others (Connolly *et al.*, 1994). Decoctions of the leaves are used as a remedy for cholera and have been found to exhibit antifertility effects in rats (Thakur *et al.*, 2010; Devi *et al.*, 2015). The essential oil of *A. odoratissimus* has shown excellent to good antihelmintic property against tape worms, earth worms and round worms (Joshia *et al.*, 2011; Singh and Sahai, 1996). Its flowers are used in the treatment of vomiting, biliousness, blood and heart diseases, itching, sweating, foul breath, thirst and headache (Sidhiqui and Garg, 1990; Vaidya and Devasagayam, 2007). The plant leaf of *Artabotrys odoratissimus* have a major contribution in the treatment of Cardiac stimulant, uterine stimulant, muscle relaxant (Brain and Bristol, 1975).

MATERIALS AND METHODS

Collection of plant material and extraction

Stem bark of *Artabotrys odoratissimus* was collected from Chittoor district of Andhra pradesh and was identified and authentified by Dr. Madhavachetty, plant taxonomist, Asst.prof, Dept. of Botany. The plant voucher No. is 0823 dated 17-12- 2018. The stem bark was allowed to dry in shade for two to four weeks. After drying, the stem bark was grinded into finely powder and stored in airtight container. The air-dried stem bark powder (100g.) was successively extracted by Soxhlet extraction with solvents of increasing polarity i.e., petroleum ether, chloroform, methanol and aqueous. The extracts were dried and stored in a sterile container for further use.

Instruments and Chemicals

Compound microscope, stage micrometer, camera lucida, drawing sheets, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica (Dissecting Microscope Lighting System-DMLS) microscope attached with Leitz (Magnification Power System- MPS) 32 camera. Solvents viz., 95% Methanol (MeOH), Petroleum ether (PE), chloroform and reagents such as phloroglucinol, glycerin, hydrochloric acid, chloral hydrate, and sodium hydroxide were procured from Merck Specialities Pvt. Ltd., Balanagar, Hyderabad, Telangana.

Pharmacognostic Studies

Fresh stem bark was taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters, physicochemical parameters and phytochemical investigation. For the microscopical studies, transverse sections of stem bark was prepared and stained as per standard procedure (Pandya *et al.*, 2010). The powder microscopy was performed according to the method of (Khandelwal, 2008).

The Total ash, Water soluble ash, Foreign matter, Loss on drying and Swelling index were determined according to the standard methods prescribed in Indian Pharmacopeia and also as per the WHO guidelines on quality control methods for medicinal plants materials (Gitanjali and Manikandan, 2012; Chase and Pratt, 1949). Fluorescence analyses were carried out according to the method of Chase and Pratt 1958 (Kokoshi *et al.*, 1958) and Kokoski 1995 (Crews, 1943).

Phytochemical Screening

Petroleum ether, chloroform, methanol and aqueous extracts were subjected to comparative phytochemical analysis for the presence of various secondary phytoconstituents using standard procedure described by kokate (Harborne, 1998) and (World Health Organization, 2011). The extract residues of the plant were subjected to phytochemical screen-

ing to detect the presence of various active phytocompounds like phenols, tannins, flavonoids, saponins, alkaloids, primary metabolites like carbohydrates, proteins and lipids (Raghuramulu *et al.*, 2003).

Mineral Analysis

The mineral like Copper, Iron, Magnesium, Potassium, Manganese, Vanadium, Titanium, Molybdenum and Zinc in stem bark of plant were determined using the standard methods given by NIN (Singh *et al.*, 2010).

Figure 1: ***Artabotrys odoratissimus***

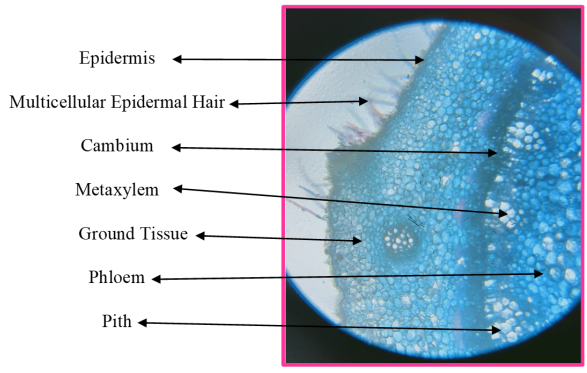

Figure 2: T.S. of ***Artabotrys odoratissimus*** **Stem.**

RESULTS AND DISCUSSION

Stem microscopy

The T.S. of young dicot stem of *Artabotrys odoratissimus* (Figure 2) shows three main zones including cortex, epidermis and stele.

Cortex

The outer part of the cortex is hypodermis middle part is general cortex, and the inner most layer is endodermis. It is present between the epidermis and stele. In the cortex the cells are arranged compactly without intracellular space.

Epidermis

This is the outermost layer formed by rectangular cells that are arranged closely without intracellular space. In the outer surface waxy layer knows as cuticle present.

Stele

It the central conducting cylinder. It is well developed. In the stele outermost layer is pericycle. Vascular bundles are arranged in the form of circle. This arrangement is called eustele. The vascular bundles are in top shaped. Cambium is present between xylem and phloem so the vascular bundles are called conjoint, collateral, open vascular bundles. In between the vascular bundles medullary rays are found.

Powder Microscopic Characteristics

The stem shows fragments of cork cells, fibers, and parenchymatous cells.

Physicochemical parameter

Physicochemical analysis of stem bark powder viz. Total ash, Water soluble ash, Foreign matter, Loss on drying and Swelling index are presented in Table 2.

Fluorescence Analysis

The fluorescence analysis of the stem powder was done and results are given in Table 3. The powder was treated with various reagents and the mixture was observed under UV light ($254_{nm.}$). 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.

Phytochemical Screening

The qualitative chemical tests were carried out for the identification of the different nature of phytoconstituents present in the stem of *Artabotrys odoratissimus* by standard procedures. They are usually tested for the presence of alkaloids, flavonoids, phenols, tannins, cardiac glycosides, triterpenes, steroids and saponins**.** The results were shown in Table 4.

Table 1: Scientific Classification

Scientific Classification	
Botanical Name	*Artabotrys odoratissimus*
Kingdom	Plantae
clade	Angiosperms
Order	Magnoliales
Family	Annonaceae
Genus	*Artabotrys*
Species	*Artabotrys odoratissimus*

Table 2: The various Physicochemical constant of stem of *Artabotrys odoratissimus*

S.no.	Physicochemical Constant	Stem Bark
1	Total ash (% w/w)	10.5
2	Water soluble ash (% w/w)	1.50
3	Acid insoluble ash (% w/w)	0.73
4	Water soluble (% w/w)	11.17
5	Alcohol soluble (% w/w)	6.41
6	Foreign matter (% w/w)	0.22
7	Loss on drying (% w/w)	5.28
8	Swelling index (mL)	3.42

Values are expressed as mean ± SD of six values.

Table 3: Observations of *Artabotrys odoratissimus* stem powder under visible light and UV (254nm.) light.

S. No.	Treatment	Visible light	Observation (Colour developed) at UV-254nm.
1	Only Powder (P)	Buff	Dark green
2	P + H2SO4 (1:1)	Green	Fluorescent green
3	P + 1N HCl	Brown	Green
4	P + 1N NaOH in water	Rust	Herbage green
5	P + HNO3 (1:1)	Green	Fluorescent green
6	P + 1N NaOH in Methanol	buff	Herbage green

Table 4: Phytochemical screening of different extracts of *Artabotrys odoratissimus* Stem.

Tested Group	Ether Extract	Chloroform Extract	Methanol Extract	
Alkaloids	—	—	+++	+++
Glycosides	—	—	+++	+++
Phenolic compound	—	—	+++	—
Steroids & Sterols	—	+++	—	+++
Saponins	—	—	+++	+++
Flavonoids	—	—	—	+++
Carbohydrates	—	—	—	+++
Tannins	—	—	—	—

Note:(+++) Present (—) Absent

Table 5: Mineral Analysis of *Artabotrys odoratissimus* Stem.

S.no.	Elements	formula	Concentration ppm.
1	Zinc	(Zn)	0.0225
2	Molybdenum	(Mo)	0.1612
3	Vanadium	(V)	0.3225
4	Titanium	(Ti)	1.7647
5	Cadmium	(Cd)	0.0189
6	Manganese	(Mn)	0.0219
7	Magnesium	(Mg)	1.0825
8	Potassium	(K)	0.2184

Mineral Analysis

Mineral analysis of plant materials provides an idea of the nutrient concentration and when multiplied by dry matter on the total uptake. Information on nutrient concentration (in tissues) depicts the nutritional status and is used as a diagnostic tool for advisory purposes. When compared with pre-established standard norms, it indicates whether the nutrient status is in the deficiency, optimum or excessive range. The results can be used to take corrective measures. All the determinations were done in triplicates and results given in Table 5.

Ethnomedically, the stem bark of this plant was used by local people in the treatment of various disease conditions without standardization (Kapoor *et al.*, 2011; Rajeswari *et al.*, 2011). The standardization of a crude drug is an integral part for establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostical parameters and standards must be established (Lachumy *et al.*, 2010). Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials (Subramanian *et al.*, 2011).

The Pharmacognostical standards for stem bark of *Artabotrys odoratissimus* is carried out for the first time in this study. The microscopical characters of the stem bark can serve as diagnostic parameters. Microscopical studies indicated the presence of median large size vascular bundle in stem. Presence of cortical vascular bundle, patches of pericyclic fibers and brown pigment containing cells are the characteristics of the plant. Ash values and extractive values can be used as reliable aid for detecting adulteration. These studies help in the identification of the plant materials. (Morton, 1949)

CONCLUSIONS

In conclusion, The quantitative phytochemical investigation gave valuable information about the different phytoconstituents present in the stem of *Artabotrys odoratissimus* , which helps the future investigators regarding the identification and preparation of a monograph of this plant part for further research. This study play a crucial role in standardization of the stem material, isolation of phytoconstituents, pharmacological investigations.

ACKNOWLEDGEMENT

Authors acknowledges the recognition of Annamalai University to provide the facility to carry out this work. We also want to support our Pharmacognostic faculty who provide their continuous support during the study.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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