OUTREACH Volume IX 2016 232 – 240
A Multi - Disciplinary Refereed Journal
ISSN (Print) : 0975 1246
ISSN (Online) : 2321 8835
www.voccoutreach.in

# Pharmacochemical Characterization of *Aristolochia* spp (Aristolochiaceae)

**A. Agnel Ruba and V. R. Mohan*,** Ethnopharmacology Unit, PG and Research Dept. of Botany, V.O.C. College, Thoothukudi *Email: vrmohanvoc@gmail.com

## Abstract

The present study has been carried out to evaluate the pharmacochemical characterization of the whole plants extracts of *Aristolochia krisagathra* and *Aristolochia bracteata*. Physicochemical parameters (Ash value and extractive value; fluorescence analysis) and phytochemical analysis were done by using the standard methods. The total ash value of whole plants of *A.krisagathra* and *A.bracteata* was found to be 10.24 % and 9.68% respectively. The extractive value of whole plants of *A.krisagathra* and *A.bracteata* in various solvents were studied. Preliminary phytochemical screening of whole plants showed the presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein. The pharmacochemical characterization will be helpful to study the active principles using modern techniques in the later part of this work.

## Introduction

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material. Adulteration or substitution is nothing but replacement of original plant with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification (Sumitra Chanda, 2014).

The genus *Aristolochia* finds a prominent place in different Indian Systems of Medicine. The different ethnic communities in India have used different species of *Aristolochia* in the treatment of various human ailments. Kanikkar tribals of Kalakad-Mundanthurai Tiger Reserve Sanctuary, Tamil Nadu, boiled equal quantity of fresh root and leaves of *A.krisagathra* in coconut oil for about 15-20 minutes over a low flame. The oil is filtered after cooling and applied on the head once in a day as the treatment of rheumatism. The therapy is used to reduce excessive heat of the body (Sutha *et al*., 2010). *A.bracteata* is used in traditional medicine as gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi *et al*., 2003). The whole plant was used as purgative, anthelmintic, antipyretic and antiinflammatory agents (Kadam *et.al*., 2012)

In spite of the numerous medicinal uses attributed to the abovesaid plants, pharmacochemical and phytochemical information about these plants will play important role in pharmaceutical industry. Hence, the current investigations describe various pharmacochemical parameters like, ash value, extractive value, fluorescence analysis and phytochemical screening of whole plants of *A.krisagathra* and *A.bracteata*.

## Materials and Methods

### Collection and Processing

The whole plant of *Aristolochia krisagathra* Sivarajan and Pradeep was collected from the natural forest of Kalakad- Mundanthurai Tiger Reserve, Western Ghats, Tirunelveli, Tamil Nadu, India. The whole plant of *Aristolochia bracteata* Retz. Was collected from Vdavalli Coimbatore, Tamil Nadu, India. The plants were identified with the help of local flora and authenticated in Botanical Survey of India, Sourthern circle, Coimbatore, Tamil Nadu, India. Voucher specimens were preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin. Tamil Nadu for further references. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

### Determination of physicochemical parameters

Determination of physicochemical parameters, such as ash and extractive values were done following the methods of African Pharmacopoeia (1986) and

Anonymous (1996). The behavior of the powdered leaf with different chemical reagents was studied and the fluorescence character was observed under UV light (Chase and Pratt, 1949).

**Preparation of extracts for phytochemical screening**

Freshly collected whole plant of *A.krisagathra* and *A.bracteata* were dried in shade, and then coarsely powdered separately in a willy mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the ethanol extracts were concentrated in a rotary evaporator. The concentrated extracts were used for phytochemical screening.

**phytochemical screening**

All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures with little modification (Brindha *et al*., 1981; Gowari and Vasantha, 2010; Shajeela *et al*., 2012)

**Results**

**Ash and extractive values**

The results of the ash and extractive values of *A. krisagathra* and *A. bracteata* whole plants are depicted in Table 1. The total ash content of the powdered whole plants of *A. krisagathra* and *A. bracteata* are 10.24% and 9.68% respectively. The present study revealed that the extractive values of the water extracts are more for both the investigated plants than for other solvent extracts studied.

**Fluorescence analysis**

The results of fluorescence analysis of *A. krisagathra* and *A. bracteata* whole plants are shown in Table 2 and 3 respectively. The whole plant powder of *A. krisagathra*, as such, fluoresced light green under day light and short UV light (254 nm) and dark green under long UV light (365 nm). The whole plant powder of *A. bracteata*, fluoresced green under day light and short UV light (254 nm) and dark green under long UV light (365 nm). The whole plant powder of *A. krisagathra* emitted the characteristic fluorescent green colour when treated with 1N HCl, concentrate HCl, 50% sulphuric acid, 40% sodium hydroxide + 10% lead acetate and chloroform. The whole plant powder of *A. bracteata* emitted the characteristic fluorescent green colour when treated with 50% sulphuric acid, 50% nitric acid, 40% sodium hydroxide + 10% lead acetate and ammonia.

**Preliminary Phytochemical Screening**

The distribution of different phytochemical constituents in petroleum ether, benzene, chloroform, methanol, ethanol and water extracts of whole plant powders of *A. krisagathra* and *A. bracteata* were evaluated qualitatively and the results are presented in Table 4. The presence of phytocompounds such as alkaloids, anthraquinones, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, sugars, glycosides and xanthoprotein have been confirmed in the methanol and ethanol extracts of both the selected plants.

**Discussion**

**Pharmacochemical Characterization**

**Physicochemical constituents**

**Ash values**

The physicochemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (African Pharmacopoeia, 1986). The total ash is particularly important in the evaluation of purity of drugs, i.e, the presence or absence of foreign organic matter such as metallic salts and / or silica (Musa *et al.,* 2006). Acid insoluble ash reflects the calcium oxalate content of the drug. In the present investigation, a considerable amount of total ash (10.24% and 9.68%) was noticed in whole plants of *A. krisagathra* and *A. bracteata* and this finding can be employed as a quality parameter to *A. krisagathra* and *A. bracteata* biomass for any adulteration. These ash values are the indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. The experimental plant samples have more water soluble ash than the acid insoluble ash. The ash value is generally an index of the purity as well as identity of the drug.

**Fluorescence analysis**

Many phytocompounds fluoresce when suitably illuminated. The fluorescent colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analysis over a satisfactory concentration range without several time consuming dilution steps prior to the analysis of pharmaceutical samples (Pimenta *et al*., 2006). The whole plant powder of *A. krisagathra* fluoresced light green under day light

and short UV light (254 nm) and dark green under long UV light (365 nm). Similarly the whole plant powder of *A. bracteata* fluoresced green under day light and short UV light (254 nm) and dark green under long UV light (365 nm).

**Phytochemical studies**

The medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents (Nostro *et al*., 2000). Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al*., 2007). Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for the detailed phytochemical investigations**.** Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening; chemo-profiling and marker compound analysis using modern analytical techniques.

The preliminary phytochemical screening of the whole plants methanol and ethanol extracts of *A. krisagathra* and *A. bracteata* revealed the presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, quinine, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein in them. The presence of these useful secondary metabolites could make these plants useful for treating different human ailments and thus providing a potential drug for human use. This is because; the pharmacological activity of any plant is usually traced to a particular phytocompound.

**Conclusion**

Standardization is an essential analytical aspect for the study of identity, purity and quality of crude drug sample of plant origin. Chemical and physicochemical analyses revealed useful information which is almost important for the quality control of whole plants to be used as crude drugs. Documentation of standardized parameters therefore are an indispensible element in the development of herbal drugs from raw plant drugs (Crude preparations), considering their desired therapeutic and safely profile.

**References**

Sumitra Chanda, 2014. Importance of pharmacognostic study of medicinal plants: An overview, *J. Pharmacog. Phytochem.* 2: 69-73.

Sutha, S., V.R. Mohan., S. Kumaresan., C. Murugan and T. Athiperumalsamy. 2010. *Ind. J. Trad. Know*. 9: 502 – 509.

Negi, P.S., C. Anantharamakrishnan and G.K. Jeyaprakash. 2003. *J.Med. Food*. 6: 401 – 403.

Kadam, M.B., S.S. Deokule, S.J. Chavan and C.V. Murumkar. 2012. *J. Nat. Prod. Plant Resour*. 2: 589 – 592.

Anonymous, 1996. Indian Pharmacopoea, Vol. I & II. Government of India, Ministry of Health and Family Welfare. The Controller of Publications, Civil Lines, Delhi – 110054.

Chase, C.R. and R. Pratt. 1949. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. American. Pharm. Associ. Sci.* **28**: 324 - 331.

Brindha, P., P. Sasikala and K.K. Purushothaman. 1981. Pharmocognostic studies of Merugan kizhangu. *Bull. Med. Eth. Bot. Res*. 3: 84-96.

Gowri, S.S. and K. Vasantha. 2010. Phytochemical screening and antibacterial activity of *Syzygium eumini* (L.) (Myrtaceae) leaves extracts. *Int. J. Pharm. Tech. Res*. 2: 1569-1573.

Shajeela, P.S., V. Kalpanadevi and V.R. Mohan. 2012. Potential antidiabetic, hyperlipidaemic and antioxidant effects of *Nymphaea pubescens* extract in alloxan induced diabetic rats. *J. App. Pharmaceu. Sci*. 2: 83-88.

African Pharmacopoeia. 1986. General Methods for Analysis Ist ed. 2: (OAU/STRC) Lagos. p.123.

Musa, K.Y., A.U. Katsayal, A. Ahmed, Z. Mohammed and U.H. Danmalam. 2006. Pharmacognostic investigation of the leaves of *Gisekia pharmacioides*. *Afri. J. Biotech*. 5**:** 956 - 957.

Pimenta, A.M., M.C. Montenegro, A.N. Ara Ujo and J.C. Martinez. 2006. Application of sequential injections analysis to pharmaceutical analysis. *J. Pharm. Biomed. Anul*. 40: 16- 34.

Nostro, A., M.P. Germanò, V. D'angelo, A. Marino and M.A. Cannatelli. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol.* 30: 379-384.

Krishnaiah, D., R. Sarbatly and A. Bono. 2007. Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnol. Mol. Biol. Rev.* 1: 97-104.

**Table 1: Ash values of the powdered whole plants of *Aristolochia krisagathra* and *Aristolochia bracteata***

| S. No | Types of ash | % of Ash values | |
|---|---|---|---|
| | | ***A. krisagathra*** | ***A. bracteata*** |
| 1 | Total ash value of powder | 10.24% | 9.68% |
| 2 | Water soluble | 4.98±0.08 | 3.51±0.03 |
| 3 | Acid insoluble ash | 3.79±0.01 | 2.11±0.01 |
| 4 | Sulphated ash | 10.26±0.78 | 11.36±0.65 |
| **Extractive values** | | | |
| **S.No** | **Name of the extract** | **Extractive Values (%)** | |
| | | ***A. krisagathra*** | ***A. bracteata*** |
| 1 | Petroleum ether | 6.89±0.04 | 5.36±0.06 |
| 2 | Benzene | 5.39±0.11 | 5.74±0.07 |
| 3 | Chloroform | 6.79±0.32 | 5.79±0.03 |
| 4 | Acetone | 6.42±0.24 | 7.96±0.12 |
| 5 | Methanol | 8.59±0.36 | 7.38±0.31 |
| 6 | Ethanol | 8.79±0.17 | 7.20±0.15 |
| 7 | Water | 10.15±0.26 | 9.80±0.17 |

* All values are mean of triplicate determination.

**Table 2: Fluorescence analysis of powdered whole plant of *A. krisagathra***

| S. No | Experiments | Visible/ day light | UV –light | |
|---|---|---|---|---|
| | | | 254 nm(short wave length) | 365 nm (long wavelength) |
| 1 | Powder as such | Light green | Light green | Dark green |
| 2 | Powder+1NNaOH(Aqueous) | Yellowish green | Yellowish green | Dark green |
| 3 | Powder+1N NaOH(Alcohol) | Greenish yellow | Green | Brown |
| 4 | Powder +1N HCL | Light green | Fluorescent green | Brownish black |
| 5 | Powder +Conc. $H_2SO_4$ | Reddish brown | Yellowish green | Dark brown |
| 6 | Powder +50% $H_2SO_4$ | Green | Fluorescent green | Dark green |
| 7 | Powder +Conc.$HNO_3$ | Reddish brown | Yellowish green | Dark brown |

| S. No | Experiments | Visible/ day light | UV –light | |
|---|---|---|---|---|
| | | | 254 nm(short wave length) | 365 nm (long wavelength) |
| 8 | Powder +Conc.HCL | Light brown | Fluorescent green | Dark brown |
| 9 | Powder +50%HNO3 | Dark green | Fluorescent green | Greenish brown |
| 10 | Powder +40%NaOH + 10% Lead acetate | Light green | Fluorescent green | Dark brown |
| 11 | Powder +Acetic acid | Greenish brown | Greenish yellow | Dark green |
| 12 | Powder +Ferric chloride | Pale green | Green | Dark brown |
| 13 | Powder +$HNO_3$ +$NH_3$ | Reddish green | Greenish yellow | Reddish brown |
| 14 | Powder +$HNO_3$ | Light green | Green | Dark green |
| 15 | Powder +Benzene | Yellowish green | Greenish yellow | Dark green |
| 16 | Powder +Petroleum ether | Light green | Leafy green | Dark green |
| 17 | Powder +Acetone | Leafy green | Greenish yellow | Dark green |
| 18 | Powder +Chloroform | Green | Fluorescent green | Dark green |
| 19 | Powder +Methanol | Leafy green | Light green | Brown |
| 20 | Powder +Ethanol | Green | Greenish yellow | Dark brown |

* All values are mean of triplicate determination

**Table 3: Fluorescence analysis of powdered whole plant of *A. bracteata***

| S. No | Experiments | Visible/ day light | UV –light | |
|---|---|---|---|---|
| | | | 254 nm(short wave length) | 365 nm (long wavelength) |
| 1 | Powder as such | Green | green | Dark green |
| 2 | Powder+1NNaOH(Aqueous) | Light Yellow | Yellow | Yellowish green |
| 3 | Powder+1N NaOH(Alcohol) | Reddish yellow | Yellowish Green | Reddish green |
| 4 | Powder +1N HCL | Pale yellow | Light yellow | Pale yellow |
| 5 | Powder +Conc. $H_2SO_4$ | Brownish red | Dark brown | Brownish black |
| 6 | Powder +50% $H_2SO_4$ | Green | Fluorescent green | Leafy green |
| 7 | Powder +Conc.$HNO_3$ | Pale yellow | Greenish yellow | Dark green |
| 8 | Powder +Conc.HCL | Pale yellow | Fluorescent green | Dark green |
| 9 | Powder +50%HNO3 | Pale yellow | Greenish yellow | Dark green |

| S. No | Experiments | Visible/ day light | UV –light | |
|---|---|---|---|---|
| | | | 254 nm(short wave length) | 365 nm (long wavelength) |
| 10 | Powder +40%NaOH + 10% Lead acetate | Pale yellow | Fluorescent green | Reddish green |
| 11 | Powder +Acetic acid | Pale yellow | Yellowish green | Mustard yellow |
| 12 | Powder +Ferric chloride | Pale yellow | Yellowish Green | Reddish brown |
| 13 | Powder +$HNO_3$ +$NH_3$ | Pale yellow with red spots | Greenish yellow | Leafy green |
| 14 | Powder +$HNO_3$ | Pale yellow | Fluorescent green | Reddish green |
| 15 | Powder +Benzene | Yellowish red | Yellowish green | Dark green |
| 16 | Powder +Petroleum ether | Yellowish red | Yellowish green | Leafy green |
| 17 | Powder +Acetone | Pale yellow | Yellowish green | Greenish yellow |
| 18 | Powder +Chloroform | Brownish yellow | Yellowish green | light green |
| 19 | Powder +Methanol | Pale yellow | Greenish yellow | Yellowish green |
| 20 | Powder +Ethanol | Pale yellow | Yellowish green | Yellowish green |

**Table 4: Preliminary phytochemical screening of whole plant of *A. krisagathra* and *A. bracteata***

| Test | Petroleum ether | | Benzene | | Ethyl acetate | | Methanol | | Ethanol | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AKW | ABW | AKW | ABW | AKW | ABW | AKW | ABW | AKW | ABW | AKW | ABW |
| Alkaloid | - | - | - | - | - | - | + | + | + | + | + | + |
| Anthraquinone | - | - | - | - | - | - | + | + | + | + | - | - |
| Catechin | - | - | - | - | - | - | - | - | - | - | - | - |
| Coumarin | - | - | - | - | - | - | + | + | + | + | - | - |
| Flavonoid | - | - | - | - | - | - | + | + | + | + | + | - |
| Phenol | - | - | - | - | - | - | + | + | + | + | - | - |
| Quinone | - | + | + | + | ++ | + | - | + | + | + | - | - |
| Saponin | - | - | - | - | - | - | + | + | + | + | + | - |
| Steroid | + | + | + | + | + | + | + | + | + | + | - | - |
| Tannin | - | - | - | - | - | - | + | + | + | + | + | + |
| Terpenoids | + | + | + | _ | + | + | + | + | + | + | - | + |
| Sugar | - | - | - | - | - | - | - | + | + | + | + | + |
| Glycoside | + | + | + | + | + | + | + | + | + | + | - | + |
| Xanthoprotien | - | - | - | - | - | - | + | + | + | + | - | - |

**AKW -** *Aristolochia krisagathra* whole plant; **ABW -** *Aristolochia bracteata* whole plant

**+ Presence – Absence**