# Pharmacognostical studies of anti-leprosy plant *Aristolochia bracteolate* retz (aristolochiaceae)

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## ABSTRACT

*Aristolochia bracteolate* Retz. (Family: Aristolochiaceae) are important constituents among 22 plants/ parts used in the perpetration of an anti-leprosy drug "SULAK". The latter is also used in the preparation of "KALMEGH"- an Ayurvedic drug. Pharmaco-chemical data available on *A. bracteolate* are scanty and totally lacking on the other. The present paper deals with pharmaco-chemical evaluation of the species with the following parameters: organoleptic, microscopic and fluorescence evaluation, estimation of biochemical and geochemical and determination of active principle and physical constants. Since the above species are used in the perpetration of the anti-leprosy drug, their chemical identity is compared with that of standard allopathic anti-loprosy agents like clofazimine, dapsone and riampicin by chemical reaction (IP method) to assess their activity equivalence.

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## INTRODUCTION

*Aristolochia bracteolate* Retz. belongs to the Family Aristolochiaceae are the important constitutes among 22 plants used in the preparation of an anti-leprosy drug "SULAK" (1–3). The pharmacognostical characters of *A. bracteolate*, used in the preparation of an Ayurvedic drug "KALMEGH" (4–7) have been to certain extent studies by (8), but no information is available on the other species.

The present study deals with the pharmaco-chemical characterisation of *A. bracteolate*. A chemical identity comparison is made with standard allopathic anti-leprosy agents like clofazimine, dapsone and rifampicin to assess their activity equivalence to treat leprosy by adopting Indian pharmacopoeal (IP) methods.

## MATERIALS AND METHODS

The plant of *A. bracteolata* were collected at their flowering from Coimbatore. Herbaria were deposited in the botany department herbarium, Bahrathiar University, Coimbatore. Whole plant species are fixed in FAA for free hand sections. The sections are stained in safranin and fast green (9) for anatomical studies. The plants were powdered with electric pulvarizer and sieved through 40mesh sieves for powder analysis.

The fluorescence studies are made according to (10–11) the procedure recommended by (12) and Indian pharmacocopia are followed for the determination of ash values and extractive values. For estimation of protein and free sugar, the methods describes by (13–14) are followed. For geochemical studies triple acid digestion of the powder and atomic analyzer are used. Histochemical location is done according to the methods described by (15–18). For preliminary phytochemical studies were made as by describes in experiments in pharmaceutical science by (19) are followed.

## OBSERVATION AND RESULTS

### *Macroscopic characters*

Leaves alternate, cordaterniform, 1.2–4.1 × 1.5–3.7cm. cordate to sagittate at base, entire, obfuse, glaucous beneath. Bracts orbicular. Flowers solitary. Perianth tube green; lipped, dark purple, 1 lipped, stamens 6, stigmatic lobes 6, glandular hairy. Capsules glabose, 10 ribbed. Seeds many cordate with rounded glands.

### *Vegetative features*

Preparation from whole part: Cylinderical; colour dark brown; fissures absent; highly flexible; more fibrous; aromatic.

### *Microscopic characters*

*A. bracteolate* (stem) it is angled in outline. Epidermis is single layered and formed of elongated cells. Primary

corex is formed of parenchymatous cells. The cells are rich in starch grains. Cork orginates from sub-epidermal region. It has alternate layers of thin walled cubical cells. Endodermis is less prominent. Large gabs are seen in the pericyclic sclerenchyma. Phloem are crystals. Xylem has large vessels and pitted. The pith is formed of thin walled parenchymatous cells. Medullary rays are broad. Secretory cells with oil and crystals in cluster are seen.

### *Leaf*

Epidermal cells are polygonal silicified cells are seen in upper epidermis stomata is of paracytic type and its index is 5.13–7.25. small group of palisade cells are horizontally divided by transverse wall. Veins are provided with greenish sheath secretory cells in mesophyll show yellowish contents. Crystals occur in mesophyll and epidermil cells.

### *Fluorescence evaluation*

The fluorescence property of the powders and their extracts of A. bracteolate as such and after treatment with different chemical reagents under day and UV light are summarized in Tables 1, 2, and 3.

### *Biochemical evaluation*

The biochemical are estimated in percentage on dry weight basis. The values obtained are average of three explicative (Tables 4 and 5).

**Table 1. Behaviour of *A. bracteolata* (whole plant) with chemical reagents.**

| S.No | Treatment of powder with under day light | Colour |
|---|---|---|
| 1 | Powder such as | Deep grey |
| 2 | N NaoH (aqueous) | Copper leaf |
| 3 | N NaoH (in methanol) | Copper leaf |
| 4 | Picric acid saturated solution | Pistachio green |
| 5 | Acetic acid (Conc) | Truck brown |
| 6 | Hcl (Conc) | Oliver green |
| 7 | $HNO_3$ (Conc) | Copper leaf |
| 8 | $H_2So_4$ (Conc) | Deep green |
| 9 | Soliwanoff's reagent | Sand stone |
| 10 | Ferric chloride 5%solution | Deep green |
| 11 | 40% NaoH (aqueous) + 10% lead acetate | New oliver green |
| 12 | Iodine solution (5%) | Deep green |
| 13 | Sadan III (in ethanol) | Brown |
| 14 | $HNO_3$ (Conc) + Ammonia solution | Gold brown |

### *Geochemical evaluation*

The minerals are estimated in parts per million (ppm) from the plant powders (Table 6)

### *Phytochemical test*

The inference obtained from screening the plant powders for their active principales are presented in the table 8.

**Table 2. Behaviour of powder of *A. bracteolata* (whole plant) with chemical reagent under UV light**

| S.No | Treatment of powder with | Colour under UV light | | |
|---|---|---|---|---|
| | | 200–280nm | 280–320nm | 320–400nm |
| 1 | Powder such as | Lt. adm. Grey | Sand stone | Dark bs grey |
| 2 | Nitrocellulose in amylacetate | New oliver green | New oliver green | Deep green |
| 3 | N naoH (aqueous) | Jede green | Oliver green | Deep green |
| 4 | N HaoH (aqu)+ Nitrocellulose (in amylacetate) | New oliver green | New oliver green | Copper leaf |
| 5 | N NaoH (in Methanol) | Dark bs grey | Deep green | Deep green |
| 6 | NaoH (in met) + Nitrocellulose (in amylacetate) | New oliver green | New oliver green | Deep green |
| 7 | N Hcl | Smoke grey | Dark bs grey | Sand stone |
| 8 | N Hcl + Nitrocellulose (in amylacetate) | New oliver green | New oliver green | Deep green |
| 9 | 50% $HNO_3$ | Oliver green | Oliver green | Sand stone |
| 10 | 50% $H_2So_4$ | New oliver green | Bus green | Deep green |
| 11 | Methanol | T.A. grey | Air gruft grey | Lt bus grey |
| 12 | Saliwanoff's reagent | Brown | Deep green | brown |

**Table 3. Behaviour of powder extractive of *A. bracteolata* (whole plant) under day and UV light**

| Name of the extractives | Day light | Colour under UV light | | |
|---|---|---|---|---|
| | | 200–280nm | 280–320nm | 320–400nm |
| Petroleum ether | Mid buff | Pistachio green | Opaline yellow | Golden green |
| Solvent ether | Deep green | Crimson red | Crimson red | Crimson red |
| Benzene | Copper leaf | Peal green | Copper leaf | Brown |
| Chloroform | Deep green | Crimson red | Crimson red | Crimson red |
| Acetone | Deep green | Opaline green | Opaline green | Crimson red |
| Ethyl acetate | Deep green | Deep green | Deep green | Deep green |
| Ethanol | Deep green | Deep green | Oliver green | Crimson red |
| Water pH 5 | Brown | Copper leaf | Copper leaf | Brown |
| Water pH7 | Brown | Brown | Brown | Brown |
| Water pH9 | Deep green | Truck brown | New oliver | Truck brown |

**Table 4. Estimation of biochemical from *A. bracteolata* (whole plant)**

| Compound | Amount in % on dry weight basis |
|---|---|
| Sugar | 37 |
| Protein | 11.7 |
| Total free amino acid | 11.0 |
| Oil | 3.1 |

**Table 5. Distribution of free amino acids *A. bracteolata* (whole plant) in powder**

| Name of amino acid | Intensity of spot |
|---|---|
| Histidine | – |
| Serine | + |
| Lysine | – |
| Arginine | – |
| Aspartic acid | – |
| Glumatic acid | – |
| Glycine | + |
| Alanine | + |
| Theronine | – |
| Praline | 4+ |
| Thyrosine | + |
| Methionine | – |
| Valine | + |
| Tryptophane | + |
| isoleucine | + |
| Phenylalanine | – |
| Leucine | + |
| Hydroxyproline | – |

**Table 6. Mineral estimation from *A. bracteolata* (whole plant) in powder**

| Minerals name | Amount in ppm |
|---|---|
| Copper | 25.01 |
| Ferrous | 2025.00 |
| Manganese | 124.95 |
| Potassium | 84.00 |
| Sodium | 20.00 |
| Zinc | 30.08 |
| Phosphorus | 7.90 |
| Sulphur | 400.35 |

**Table 7. Phytochemical evaluation of *A. bracteolata* (whole plant) in powder**

| Active principle | Name of the test | Degree of precipitation / colouration |
|---|---|---|
| Leuco anithocyanins | – | 4+ |
| Flavones | – | + |
| Glycones | – | + |
| Aglycones | – | 7+ |
| Sterols and triterpenes | Liebermann burchard salkowski | +– |
| Anthraquinones heterosides | Borntrager BZ AZ Modified | 3+3+3+2+ |
| Saponin | BZBorntrager AZ FrothLiebermann Burchard | –7+ |
| 2–deoxy sugar | Killer–kiliani | 3+ |
| Tannin | – | – |
| Phenol | – | 7+ |
| Cardiac glycosides | Keddi reagentModifies keddi reagent | -– |

**Table 9. Physical constants of *A. bracteolata* (whole plant) in powder**

| Percentage of | | | Percentage of insoluble ash in | | | |
|---|---|---|---|---|---|---|
| **Total solid** | **Total ash** | **Total sulphated ash** | **Ethanol** | **Water** | **N NaoH** | **5% Hcl** |
| 92 | 16.7 | 17.25 | 7.25 | 6.25 | 5.70 | 1.21 |

**Table 8. Results of alkaloid screening of *A. bracteolata* (whole plant) in powder**

| Extract | Degree of precipitation | | | | |
|---|---|---|---|---|---|
| | **DROG** | **HAG** | **SCH** | **VAL** | **WAG** |
| Petroleum ether | + | + | – | – | + |
| Solvent ether | + | + | – | – | – |
| Benzene | + | + | – | – | – |
| Chloroform | + | + | – | – | – |
| Acetone | 2+ | + | – | – | + |
| Ethyl acetate | + | + | – | – | – |
| Ethanol | + | + | + | + | + |
| Water | + | + | – | + | + |

+ = indicates positive; – = indicates negative; No. + = indicates intensity of precipitation

**Table 10. Extractive values of *A. bracteolata* (whole plant) in powder**

| Name of the extract | Value in percentage |
|---|---|
| Petroleum ether | 12.33 |
| Solvent ether | 12.79 |
| Benzene | 8.73 |
| Chloroform | 14.47 |
| Acetone | 14.92 |
| Ethyl acetate | 7.77 |
| Ethanol | 16.95 |
| Water | 60.00 |

### *Extractive values*

The ash values and extractive values were tabulated in 9 and 10.

### *Chemical identity comparison*

The results of chemical identity of plant powders with that of standard sntileprosy agents like clofazimine, dapsone and rifampicin for their chemical activity equivalence by chemical reaction method (IP) are summarized in Table 11.

## DISCUSSION

The plant has high content of sugar (37%). Free amino acids like serine, glycine, alanine, prolone, tyrosine, valine, isoleucine and leucine are located. Among them praline is rich. Geochemical analysis reveals the richness of ferrous (2025ppm) and sulpher (400.35ppm) in the plant. The maximum alkaloids precipitation reaction with its precipitating reagent is observed from water and ethanol extractive.

Total solid and ash content of the sample is 92% and 16.70% respectively. The ash insolubility is less in 50% Hcl and light in ethanol. Sample gives high extractive values (60%) in water.

The chemical reaction comparison with anti leprosy agents showes activity equivalent to the presence of clofozimine (4.1%), dapsone (9.3%) and rifampicin (2.1%). When chemical identity of plant powders are compared with that of standard anti-loprosy agents, it is

**Table 11. Chemical comparison of *A. bracteolata* (whole plant) powder with antileprosy agents.**

| Antileprosy agents | Preliminary test | | | | Confirmatory test by titration% ob=n dry wt. basis |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | |
| Clofazimine | 4+ | 3+ | 0 | 0 | 4.1 |
| Dapsone | 3+ | 4+ | 4+ | 5+ | 9.3 |
| Rifampicine | 3+ | – | – | – | 2.1 |

* the values are average of three replicatives
ABCD - colorations/precipitation reaction as per ID method
Dapsone - have ABCD reactions/tests.
Rifampicin - have a only

found that the powders follow similar reaction to that of the standard. The chemical activity equivalence by chemical reaction reveals that *A. bracteolate* is slightly more close to the anti-leprosy agents. However, their efficacy in leprosy treatment will be confirmed by our pharmacological studies.

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