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Review Article

A review on ethnopharmacological utility, traditional knowledge and phytochemistry of *Aristolochia* species in Assam, India

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Abstract

Aristolochia L. (Aristolochiaceae) is widely used throughout South-East Asia for the treatment of several diseases. Different species of this genus are known by similar local names in Assam. This review aims to provide up-to-date information on *Aristolochia* species distributed in Assam, including its traditional uses, phytochemical and pharmacological properties, in exploring future therapeutic and scientific potentials. The information on ethnobotany, phytochemistry and pharmacological aspects were collected by performing literature searches. Assam hosts a total of six species of *Aristolochia*. The taxonomy and distribution are presented. Traditionally the tubers are used by the local people to treat stomach pain, malaria, dysentery, high blood pressure, body pain, urinary tract infections, headache, impotency etc. It has considerable pharmacological properties including antimicrobial, antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-fertility, anti-venom, anti-diarrhoeal, anti-pruritic, anti-feedant and toxicological activities. Approximately a total of 200 compounds have been isolated from these species. So far, pharmacological investigations are only done on three *Aristolochia* species, whereas the other three are simultaneously used for the same purposes. Most of the medicinal properties attributed to these *Aristolochia*, have not yet been investigated and proven under a scientific study. This highlights the importance of *Aristolochia* as a valuable candidate for future studies.

Keywords: *Aristolochia*; bioactive compounds; distribution; flora of Assam; pharmacology; taxonomy; traditional knowledge

Introduction

Medicinal plants serve humans as a great source of therapeutics and pharmaceutical manufacturing. The practices of using medicinal plants in the treatment of common diseases are part of the traditional knowledge among the different communities throughout the world. The dependencies of the traditional communities on the naturally occurring herbs are due to better cultural acceptability, compatibility and adaptability of the

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plants with the human body and lesser side effects (Gupta *et al*., 2010; Oladeji, 2016). Research carried out during the past few years have resulted in the isolation of more than a thousand bioactive compounds from medicinal plants having disease-preventing properties as antioxidants, detoxifying agents, immunity-potentiating agents and neuropharmacological agents (Saxena *et al.,* 2013).

The increasing demands of herbal medicine in the 21st century in both developed and developing countries indicate the public interest in traditional, complementary and alternative medicines. There is a belief that herbal medicines provide long-lasting healing, minimal adverse effects, lesser cost, well-practiced knowledge and promote healthier living in contrast to the adverse effects of allopathic drugs (Gupta *et al.,* 2010). In rapidly developing countries such as India and China, the role of plant-derived medicine in the health care system is about 80% (Khan, 2016). The active phytoconstituents may be a mixture of secondary metabolites like alkaloids, saponins, tannins, glycosides, phenols and flavonoids etc. The extraction, isolation, detection and identification of such phytochemicals are necessary for establishing the quality control, mechanism of their action on the body, safety and efficacy (Saxena, 2013; Ogunmefu, 2018).

The genus *Aristolochia* L. (Aristolochiaceae) is widely distributed in tropical to temperate regions throughout the world (Hwang *et al.,* 2003). It is the largest genus in the family accounting for about 534 accepted species (POWO, 2019), of which India is represented by 20 species (Borah *et al.,* 2019; revised). They are mostly perennial climbers, with ovate cordate leaves and fusiform rhizomes. It can be differentiated from its other congeners (*Saruma* Oliv., *Thottea* Rottb. and *Asarum* L.) by a combination of several characters such as woody or herbaceous habit, axillary flowers arranged in fascicles or solitary, uniseriate perianth, connate carpels and dry capsules.

Among the 20 species of *Aristolochia* distributed in the country, six of them are presently reported growing wild from the state of Assam (Borah *et al.,* 2019). *Aristolochia indica* L. is found in Lower Assam, *A. cathcartii* Hook.f. is distributed towards the both the banks of the River Brahmaputra in Upper Assam, whereas *A. saccata* Wall. is towards its South bank (doubtful). *A. platanifolia* (Klotzsch) Duch., *A. assamica* D. Borah & T.V. Do towards the foothills of Arunachal Pradesh in Upper Assam, on either side of the Brahmaputra basin and *A. acuminata* Lam. (syn: *A. tagala* Cham.) is found throughout the region and is the most widespread species, among its congeners (Figure 1). *A. cathcartii, A. platanifolia* and *A. saccata* falls under the subgenus *Siphisia* and can be differentiated from all other species by their strongly curved perianth, U- or horseshoe shaped tube and a 3-lobed gynostemium. *A. cathcartii* and *A. saccata* are very close allies and are often confused, *A. cathcartii* is recognized by rectangular limb, inner surface of limb lobes covered with bristle-like papillae and purple dotted throat vs. irregularly circular limb, papillae and dots absent in *A. saccata*. Whereas, *A. platanifolia* can be distinguished from both of them, by its dissected leaves and a bell-shaped limb. The remaining three falls under the subgen. *Aristolochia* series *Podanthemum* and subgen. *Aristolochia* series *Aristolochia*. *A. assamica* can be recognized by the absence of stipe absent between the ovary and the utricle and terete branches (series *Aristolochia*) vs. stipe present between the ovary and the utricle and branches angular or ribbed (series *Podanthemum* with the remaining two species). *A. acuminata* is distinguished by orbicular to ovate lamina with a long petiole up to 5 cm whereas, *A. indica* by obtuse-oblong to oblong-lanceolate lamina with a short petiole up to 2 cm (Do *et al*., 2015; Borah *et al*., 2019).

However, no comprehensive review of the genus has been reported from this particular region. The present review is aimed to focus on providing information about traditionally used natural medicine, phytochemistry and pharmacology of *Aristolochia* species from Assam, India. We tried summarizing the best available evidence of traditional uses, phytoconstituents and pharmacological activities regarding *Aristolochia* spp. along with structural features of some important phytoconstituents. Multiple databases and platforms Google Scholar, Scopus, PubMed, Web of Science, ResearchGate and Academia were searched for relevant studies which included multiple keywords to elicit the data on *Aristolochia*. Chemical structures were drawn using Chem Draw Ultra 8.0 software by following the PubChem database (Figure 2).

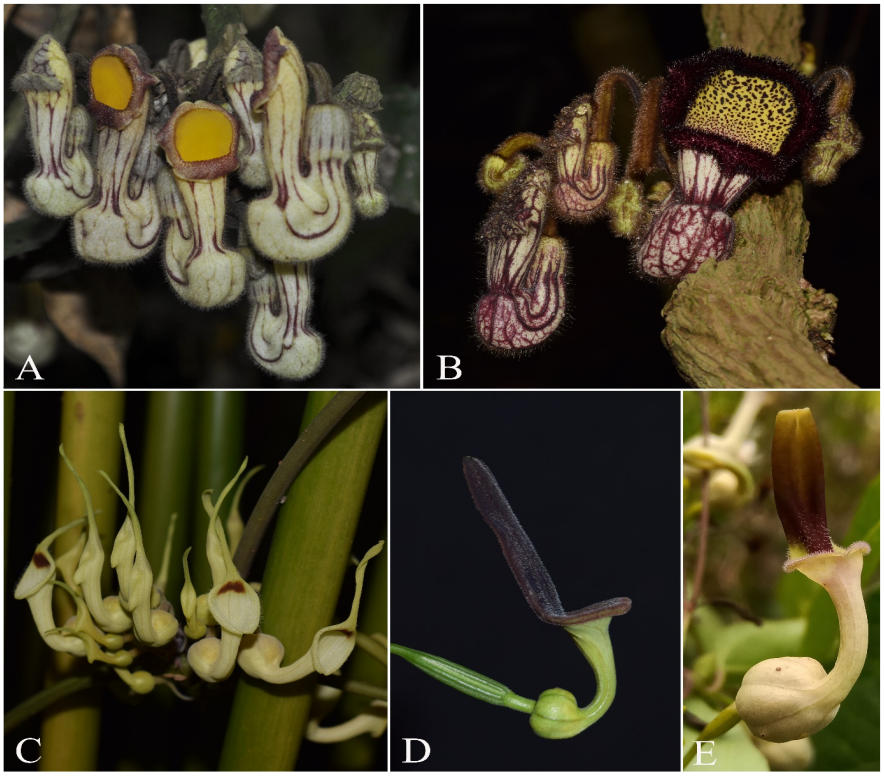


Figure 1. *Aristolochia* species distributed in Assam: A. *A. platanifolia*, B. *A. cathcartii*, C. *A. assamica*, D. *A. acuminata*, E. *A. indica*
(A by Khyanjeet Gogoi, B-D by Dipankar Borah & E by Goutam Panda)

Traditional uses of *Aristolochia* species

Aristolochia spp. has been used in traditional medicine by different communities around its occurrence for a long time. The tubers of *Aristolochia* are used against a multitude of ailments in Assam. Correlating the ethnomedicinal reports with modern pharmacological and phytochemistry studies, shows consistency with the latest findings. *A. acuminata* is used to treat diarrhoea and dysentery by several tribes residing in Assam (Rao, 2019). *A. saccata* is used to treat stomach ache, constipation, dysentery, fever, body pain, jaundice, sprains and fracture by the Karbi, Tiwa, Pnar and Bodo-Kachari people residing in Karbi-Anglong districts of Assam (Basumatary *et al.,* 2014; Teron, 2019). The population in Majuli Island and around Gibbon Wildlife Sanctuary uses its roots to treat tonsillitis, cough, piles, malaria fever and diarrhoea (Sarmah and Saikia, 2014; 2016). The roots of *A. indica* are used to heal wounds and to enhance fertility in males by the traditional healers of Dhemaji district of Assam (Taid *et al.,* 2014). It has also been reported to be used by the people of Dibru-Saikhowa Biosphere Reserve for the treatment of certain asthmatic problems and skin diseases such as leucoderma (Nath *et al.,* 2008; Purkayastha *et al.,* 2007). The *Deori*, *Muttak* and *Nepalese* community residing in the Dibrugarh district of Assam reported using the decoction of the leaves of *A. indica* to treat dysentery, diarrhoea and melena (Borah *et al.,* 2006). *A. cathcartii* is used against stomach aches, urinogenital disorders and as an insect repellent by the people in and around Manas Biosphere Reserve (Paul *et al.,* 2011 a, b). Similarly, the roots of *A. assamica* and *A. cathcartii* are used to treat stomach pain, malaria, dysentery, high blood pressure, body pain, urinary tract infections, headache and cough by the fringe people of Behali reserve forest in Biswanath district (Borah *et al.,* 2020). However, several other ethnomedicinal reports have been published for the studied species of *Aristolochia* outside Assam are shown in Table 1.

Table 1. Summary of traditional uses of the studied *Aristolochia* species outside Assam

Species	Local names	Parts used	Traditional uses	Regions	References
A. indica	*Aadagam, Aaduthinnapalai, Adgam, Aduthinnapalai', Beelieshwariballi,Bhedi Janete, Bhedijanetet (Santali), Cheriyaarayan, Chong-khengsum, Chotoishe, Eeshwariballi, Eeshwariballi, Eeswaramool, Eshwaramooli, Eshwarigida, Eswaramooleekai, Garudakodi, Ghorth, Gorisal, Ichegach, Ichharmuli, Isharmul, Ishermul, Ishwaraberu, Ishwarmon, Ishwarmul, Isramuli, Israul, Iswar, Iswaramuli, Iswari, Iswarmul, Iswarmula, Kalesar, Karakalam, Karudakodi, Kirmar, Nagasaram, Nagbel, Nalla Eshwari, Nalla Eswari, Nalleshwari, Nalleswari, Perumanthikodi, Perumarindu, Saapsun, Safed ishri, Sivan mooligai, Sunanda, Tang gwaysobawai, Thalaisuruli, Thazhaisurulikodi, Thella usiri*	Bark, Fruit, Leaf, Root, Root-bark, Stem, Tuber, Twig, Whole plant	Abortifacient, Analgesic, Anodyne, Anti-inflammatory, Antibiotic, Antidote against poison, Antihelmintic, Aphrodisiac, Asthma, Blood purifier, Bowel complaints, Burns, Cardio tonic, Cattle bloat, Cattle diarrhoea, Cattle fever, Cholera, Cold & cough, Colic, Dandruff, Diarrhoea, Diuretic, Dyspepsia, Eczema, Emmenagogue, Ephemeral, Fever, Folk belief, Gastric diseases, Gonorrhea, Hemorrhagic septicemia, Headache, Herpes, High blood pressure, Horn ablation, Injury, Insect bite, Intermittent fever, Jaundice, Leishmaniasis, Leprosy, Leucoderma, Leucorrhoea, Liver diseases, Madness, Malarial fever, Mastitis, Menstrual problems, Mosquito bite, Nervous disorders, Neuro-Tonic, Oral infection, Paralytic disease of cattle, Piles, Pneumonia, Poison bite, Pruritus, Rash, Rheumatic Arthritis, Rheumatic fever, Rheumatic pain, Scabies, Scorpion bite, Septic due to skin allergies, Sexual problems, Skin diseases, Snake bite, STD, Stomach ache, Stomach disorders, Snake repellant, Tooth-ache, Tumor, Ulcers, Unconsciousness, Uterine flow, Worm infection, Treating wounds.	India (Andhra Pradesh, Gundlabrahmeswaram Wildlife Sanctuary, Eastern Ghats, Himachal Pradesh, Jharkhand, Karnataka, Kerala, Parambikulam Wildlife Sanctuary, Madhya Pradesh, Naoradehi Wildlife sanctuary, Maharashtra, Orissa, Rajasthan, U.T of Puducherry, Tamil Nadu, Kodiakarai Reserve Forest, Uttar Pradesh, West Bengal) and Bangladesh	Basak *et al.*, 2016; Bhandary and Chandrasekhar, 2011; Bhat *et al.*, 2014; Biswas *et al.*, 2010; Bose *et al.*, 2014; Chakraborty and Bhattacharjee, 2006; Choudhary *et al.*, 2011; Das and Bondya, 2015; Das and Mondal, 2012; Devendrakumar and Anbazhagan, 2012; Dey and De, 2012; Galav *et al.*, 2013; Ganesan *et al.*, 2008; Gritto *et al.*, 2015; Hiremath and Taranath, 2010; Jain *et al.*, 2008; Jeeva *et al.*, 2006; Jeyaprakash *et al.*, 2011; Johnsy *et al.*, 2012; Kamble *et al.*, 2016; Kanneboyena *et al.*, 2015; Kingston *et al.*, 2007; Kiruba *et al.*, 2006; Kumar *et al.*, 2014; Kumar *et al.*, 2019; Marandi and Britto, 2014; Michl *et al.*, 2013; Murthy, 2012; Naturu *et al.*, 2013; Nithyadevi and Sivakumar, 2014; Nizar *et al.*, 2015; Panda and Padhy, 2008; Partha and Hossai, 2007; Poornima *et al.*, 2012; Prakasha *et al.*, 2010; Prashantkumar and Vidyasagar, 2006; Ragupathy and Newmaster, 2009; Rahmatullah *et al.*, 2013; Rajakumar and Shivanna, 2010; Rajashekharan *et al.*, 1989; Rao *et al.*, 2010; Ratnam and Raju, 2005; Reddy *et al.*, 2009; Reddy *et al.*, 2015; Rout and Panda, 2010; Sambandan and Dhatchanamoorthy, 2012; Saradha *et al.*, 2017; Sen, 2008; Senthilkumar *et al.*, 2006; Shivakumar and Parashurama, 2014; Shivanna and Rajakumar, 2011; Shukla *et al.*, 2010; Silambarasan *et al.*, 2017; Sivaperumal *et al.*, 2009; Sivasankari *et al.*, 2014; Sudeesh *et al.*, 2012; Sulochana *et al.*, 2014; Swarnalatha *et al.*, 2017; Tiwari and Yadav, 2017; Tripathi and Sikarwar, 2013; Usha *et al.*, 2015; Vijayan *et al.*, 2007; Yadav and Prakash, 2014; Yesodharan and Sujana, 2007
A. acuminata	Eswaramulli, Eachamulla, Garudakkody, Isswarmuli, Jarboporol, Khurthlong, Mala aryan, Malaiarasam, Nsoihenchi, Peru eswaramooligai, Valiyaarayan	Fruits, Leaves, Roots, Stem	Abdominal pain, Abortifacient, Analeptic, Antipyretic, Anti-inflammatory, Bone Fracture, Bilious disorders, Carminative, Diarrhoea, Dysentry, Emmenagogue, Health tonic, Loss of appetite, Malaria, Muscle relaxant, Rheumatism, Regulate menstrual disorders, Snake Bite, Stomach ache, Swollen limbs, Stimulate uterine flow, Snake and Scorpion poison, Tumour, Venereal disease	India (Arunachal Pradesh, Agasthiayamalai Biosphere Reserve, South India, Kerala, Meghalaya, Nagaland, Tamil Nadu, Trivandrum, Tripura, The Western Ghats, Tirunelveli hills), Bangladesh, Indonesia, Philippines, Thailand	Biswas *et al.*, 2010; Britto and Mahesh, 2007; Dey *et al.*, 2012; Devi Prasad *et al.*, 2013; Ignacimuthu and Ayyanar, 2005; Kayang, 2007; Bose *et al.*, 2014; Rajashekharan *et al.*, 1989; Rahman *et al.*, 2007; Reang *et al.*, 2016; Sulochana *et al.*, 2014; Silambarasan *et al.*, 2017; Santhosh Kumar *et al.*, 2019; Tripatara *et al.*, 2012

A. saccata	Batelong, Krahlahit, Rikang	Leaves, Roots, Stem, Tubers	Body pain, Diarrhoea, Dysentery, Haemorrhage, Jaundice, Tonsil	Arunachal Pradesh, Meghalaya, Nagaland	Basumatary *et al.*, 2014; Kayang, 2007; Teron and Borthakur, 2013; Rao, 2019
A. cathcartii		Leaves, Rhizome, Roots, Stem	Food poisoning, Insect repellent, Liver disorders, Promotes flow of urine, Stomach ailments	Meghalaya	Kayang, 2007; Syiem *et al.*, 2006

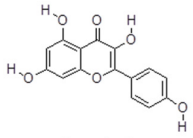
Reported phyto-compounds from *Aristolochia* species

The phytochemical analysis is must to justify the scientific accuracy in the usage of herbal medicine and unearth the basis of treating diseases efficiently. During the last couple of decades, extensive studies were done on the phytochemical constituents found in various plant species and the genus *Aristolochia* was no exception. The phytoconstituents of the genus were extensively studied and many scholars have reported numerous compounds of significant importance from the plants of this genus. Aristolochic acids and it's derivatives, aristolactams, aporphines, protoberberines, isoquinolines, benzylisoquinolines, amides, flavonoids, lignans, diphenyl ethers, coumarins, tetralones, terpenoids, benzenoids, steroids were the secondary metabolites that have been characterized from the *Aristolochia* species (Kuo *et al.,* 2012). In this section of the review, the constituents found in 3 out of 6 species of *Aristolochia* found in Assam are compiled and comprehensively presented in tabulated form (Table 2). Reliable data regarding the phytochemical constituents present in three species viz. *A. saccata*, *A. platanifolia* and *A. assamica* were not found which indicates that scientific analysis of phytochemical constituents present in those species is yet to be isolated and studied.

Table 2. Phyto-constituents of *Aristolochia* species distributed in Assam

Species	Phyto-constituents reported	Plant part	References
A. acuminata	Kaempferol	Root	Battu *et al.*, 2011
	Aristolochic acid I	Leaves	Ioset *et al.*, 2002
	Aristolochic acid A, Aristolochic acid D		Mix *et al.*, 1982
	Aristolochic acid A, Tuberosinone		Chen *et al.*, 1987
	Aristolactam C IIIa, Dehydrooxoperezinone, Pyriferine A, Isocorydine, Lagesianine A, Kaempferol, 3,5-Di- O-caffeoylquinic acid, Aristolactam IIIa; N-b-D-Glucopyranosyl isomer, Aristolactam IIIa; O-β-D-Glucopyranoside isomer, Aristolochic acid I, 4-O-beta-D-glucosyl-4-coumaric acid, Leptantine, Madolin W/K/A/R, Perillyl acetate, Beta sistosterol, Δ-13,14-2-Oxokolavenic acid, Madolin L/M/S, 3-Oxoishwarane, Aristolactone, Stigmasterol, Stigmastane-3,6-diol; (3b, 5a, 6a, 24R)-form, Diketone	Roots	Hadem *et al.*, 2019
	Aristolochic acid I, Aristolochic acid II	Root	Tripatara *et al.*, 2012
	Anthocynidin 3-glycosides and 6-hydroxylated flavonols, Chalcone glycosides	Root	Hadem *et al.*, 2016
	Aristolactam BII, Aristolactam II, Sauristolactam, Aristolactam I, Aristolactam AII, 7-methoxyaristolactam IV, 3-hydroxy-4-methoxy-10-nitrophenanthrene-1-carboxylic acid methyl ester, Ariskanin A, Ariskanin D, Ariskanin E, Aristolochic acid C, Ariskanin C, Ariskanin B, Aristolactam-N-β-Dglucoside, Cepharanone A N-β-D-glucoside	Whole plant	Liu *et al.*, 2020
A. indica	β-Caryophyllene, α-Humulene, Ishwarone, Caryophyllene oxide I, Ishwarol, Linalool, α-Terpinolene, Ishwarane, Aristolochene, Cis-3-Hexenol, Germacrene D, Octen-3-ol, 3-Hexenyl acetate, Camphor, Nonanol, Humulene oxide, Nerolidol, β-Farnesene, β-Bisabolene, Pinocarveol, δ-Cadinol, β-Elemene, α-Terpineol, β-Farnesol, Octanol, Caryophyllene oxide II, α-Bisabolol, Germacrene A, Ledol, 2- Octanol, Hexyl acetate, Thymol, Indole, β-Phellandrene, Tetradecanol, 5βH,7β,10α-selina4(14),11-diene, β-Pinene, Borneol, Terpinene-4-ol, β-Selinene, Hexanol, (12S)-7, 12-Secoishwaran-12-ol, Camphene, Tricyclene	Aerial part	Jirovetz *et al.*, 2000
	Aristololactam N-β-D-glucoside, 3β-hydroxy-stigmast-5-en-7-one, 6β-hydroxy-stigmast-4-en-3-one	Root	Achari *et al.*, 1981
	Aristolochic acid I, Aristolochic acid-D, Methyl Aristolochate, Aristolactam-A II, Aristolactam I, Aristolactam, Aristolactam-C N-β-D Glucoside, Aristolactamβ-D Glucoside	Root	Mix *et al.*, 1982
	Aristolochic acid I	Root	Kupchan and Doskotch, 1962
	Savinin, Aristolochic acid-I, (+)-ledol, (12s)- 7,12-secoishwaran- 12-ol, Aristolactam N-β-D-Glucoside, Aristolactam, Aristolactam A-II, Methyl Aristolochate, Aristolochic acid-I, Aristolochic acid-D, Cepharadione A, Aristolindiquinone, Magnoflorine	Root	Che *et al.*, 1984

	Ishwarone, Ishwarol, 5βH, 7β, 10 α-selina4(14), 11-diene, Aristolochine alkaline, Isoaristolochic acid, Allatonin, (12s)- 7, 12-secoishwaran-12-ol, Aristolactam N-β-D-Glucoside, 3β-hydroxy-stigmast-5-en-7-one, 6β-hydroxy-stigmast-4-en-3-one, Aristolindiquinone, Aristololide, 2-hydroxy-1-methoxy-4 Dibenzo quinolone-4,5-(6H)-dione, Cepharadione, AristolactamIIa, Aristolactam glycoside I, Stigmastenones II, Stigmastenones III, Methyl Aristolochate, β-sitosterol-β-D-glucoside, α-Pinene, trans-Pinocarveol, Pinocarvone		Dey *et al.*, 2011
	Astragalin, (-) hinokinin, Aristolochic acid I, Aristolactam I, Aristolochic acid II	Aerial part	Desai *et al.*, 2014
	Ishwarone		Ganguly *et al.*, 1969
	Ishwarane, Aristolochene	Root	Govindachari *et al.*, 1970
	Stigmast-5-en-3β-ol (β-sitosterol)	Aerial part	Karan *et al.*, 2012
	α-pinene, Camphene, β-pinene, p-cymene, Limonene, trans-pinocarveol, Pinocarvone, Terpinen-4-ol, Myrtenol, Myrtenal, Carvone, α -terpinyl acetate, Aromadendrene, (E)-β-ionone, α -cadinol	Stem	Kanjilal *et al.*, 2009
	Aristolochic acid-D, Aristolochic acid-n methyl ether lactam, Aristololactam β-D-glucoside	Roots	Kupchan and Meriano, 1968
	Aristolochic acid I, Aristolochic acid II, Aristolochic acid IV, Aristolochic acid D, Aristolochic acid IIIa, Aristolochic acid Ia, Cepharadione A, Aristolactam I; N-β-D-glucopyranoside, Aristolactam AII, Aristolactam III, Aristolactam I, Aristolactam IVa, Aristolactam AIII, Aristolactam II, Aristolactam II; N-β-D-glucopyranoside, Aristolactam IIIa; N-β-D-glucopyranoside, Aristolactam Ia; N-β-D-glucopyranoside, Aristoloterpenate I, Ariskanin B, 9-Methoxyaristolactam II, Norcepharadione A	Stems, Leaves	Michl *et al.*, 2013
	Aristolochic acid–A, Aristolochic acid IVa, Methyl Aristolochate, Aristolactam AII, Aristolactam I, Aristolactam, Aristolochic acid D, Aristololactam-C N-β -D-glucoside, Aristololactam N-β -D-glucoside		Mix *et al.*, 1982
	(12S)-7,12-secoishwaran-12-ol, (+)-ledol, Ishwarane	Root	Pakrashi *et al.*, 1980
	d-camphor, Sesquiterpene A, Sesquiterpene B, Ishwarane, Ledol	Root	Rao *et al.*, 1955
	Methyl ester of 12-nonacosenoic acid, n-heptadecane, ntriacontane, Palmitic acid, hexacosanoic acid, Stigmast-4-en-3-one, Friedelin, Cycloeucalenol, Rutin, Aristolactone	Root	Sati *et al.*, 2011
	(S)-Linalool, α –Terpinolene, β –Caryophyllene, Caryophyllene oxide, Caryophyllene oxide,		Wu *et al.*, 2004
A. cathcartii	Aristolactam I, Aristolactam AII, Aristolochic acid A, Aristolochic acid BII	Whole herb	Zhang *et al.*, 2016
A. saccata	-	-	-
A. platanifolia	-	-	-
A. assamica	-	-	-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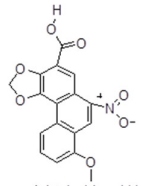
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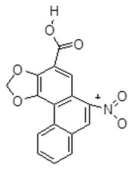
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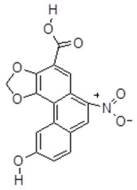
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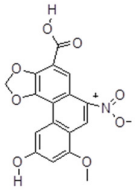
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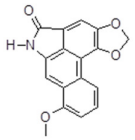
Aristolactam I

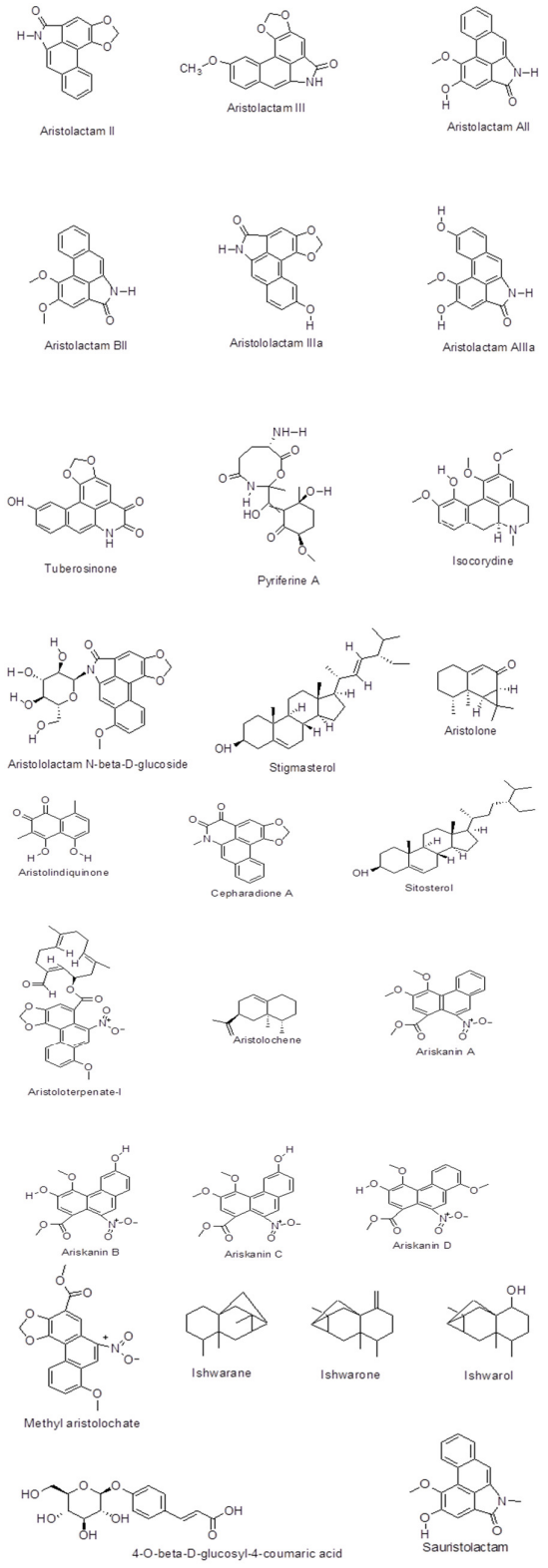

Figure 2. Chemical structures of some important Phyto-constituents of *Aristolochia* species

Pharmacological activity of reported Phyto-compounds of *Aristolochia*

Antimicrobial activity

The antibacterial activity of leaves of *A. acuminata* was studied by disc diffusion method against gram-positive *Staphylococcus lentus* and *Bacillus cereus*, gram-negative *Serratia marcescens, Candida albicans*, bacteria and fungi *Candida dubliniesis* and *Cryptococcus neoformans*. Acetone extract showed the highest inhibition zone against gram-positive organisms than against gram-negative organisms such as *Staphylococcus lentus* and *Bacillus cereus* (Hercluis *et al*., 2018).

Similarly, aerial parts of *A. indica* were also studied by disc diffusion method against *Pseudomonas aeruginosa, Basillus subtilis, Staphylococcus aureus, Escherichia coli, Bacillus sphaericus (*syn. of *Lysinibacillus sphaericus), Salmonella typhimurium*. The extracts showed a moderate antibacterial activity (Shafi, 2002). Murugan and Mohan (2012) tested against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* and found that petroleum ether, acetone and methanol plant extracts showed good results against all the tested pathogens. Venkatadri *et al.* (2015) studied the whole plant extracts by agar well diffusion method against multidrug-resistant β-lactamases producing bacteria and ethanolic extract showed minimum inhibitory concentration values of 50-100 µg/ml and 100-200 µg/ml. Naik *et al.* (2015) studied against three Gram-positive (*Staphylococcus aureus, Bacillus coagulans, B. subtilis*) and three Gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) bacteria. The leaf extract caused high inhibition of *B. coagulans* followed by *B. subtilis* and the least inhibition caused by leaf extract was recorded against *S. aureus*. In the case of flower extract, *S. typhi* and *B. coagulans* were inhibited to a higher extent when compared to other bacteria. Antifungal activity was studied by poisoned food technique against test fungi namely *Bipolaris sorokiniana* (from root rot of wheat), *Fusarium oxysporum f.sp. zingiberi* (from rhizome rot of ginger), *Colletotrichum capsici* (from anthracnose of chilli) and *Curvularia sp.* (from mouldy grains of sorghum) and the results revealed that *F. oxysporum* displayed higher susceptibility to leaf and flower extracts followed by *Curvularia sp., B. sorokiniana* and *C. capsici.* Umamaheshwari and Murthy (2012) studied against *Bacillus subtilis*, five different antibiotics namely Ciprofloxacin, Nitrofurantoin, Ofloxacin, Pefloxacin and Sparfloxacin were used as standard, results showed that the root extracts exhibited different degrees of antibacterial activity of which butanol extract of inhibition zone (2.4 cm) and ether extract (2.0 cm) showed maximum activity.

Antioxidant activity

The free radical scavenging activity of methanolic root extracts of *A. acuminata* was tested by Hadem *et al.* (2016) by DPPH method and, found significant value as compared to standard compound ascorbic acid. At 1000 µg/ml concentration, aqueous stem extract of A. indica showed higher scavenging activity of 66.66±4.67% compared to chloroform leaf extract of 48.33±3.38% in DPPH method (Subramaniyan *et al.*, 2015). Naik *et al.* (2015) found the ethyl alcohol extracts of leaves and flowers of A. indica at 100µg/ml concentration showed 48.68% and 10.52% DPPH radical scavenging activity respectively where ascorbic acid was used as standard. The aerial parts of A. indica exhibited IC50 value of 7.325 µg/ml at 25 µg/ml concentration when tested by DPPH radical scavenging method using ascorbic acid as standard and IC50 value of 8.498 µg/ml at 10 µg/ml concentration when tested by superoxide anion radical scavenging method with curcumin as standard (Karan *et al.*, 2012). Thirugnanasampandan *et al.* (2008) studied the antioxidant activities of both *A. acuminata* and *A. indica* using three solvents e.g., petroleum ether, chloroform and ethyl acetate extract. Among the extracts, the highest reducing power activity has shown by the ethyl acetate extract of *A. acuminata* (1.28%) and *A. indica* (1.01%). In Ammonium thiocyanate assay, petroleum ether (10 ml) extract of *A. acuminata* showed the highest activity of 57.42% and *A. indica* ethyl acetate extract showed the highest activity of 40.21% compared to Linoleic acid.

Anti-inflammatory activity

Ethyl acetate and ethanol extracts of *A. acuminata* roots at doses 200 and 400 mg/kg produced a significant reduction in the Carrageenan-induced paw edema on Wistar albino rats. The test samples exhibited an inhibitory effect for both COX and LOX enzymes, in in-vitro MTT colorimetric assay. Among the isolated phytoconstituents from the plant "Kaempferol" was responsible for the highest inhibition of PGE2 and LTB4 at 87.7% and 91.4% released from calcium ionophore and LPS IFN γ–stimulated macrophages than standard drug indomethacin (Battu *et al.*, 2011). Aristolactam I and (-) Hinokinin isolated from *A. indica* also exerted anti-inflammatory effects and inhibited the production of IL-6 and cytokines TNF-α in LPS-stimulated THP-1 cells (Desai *et al.*, 2014). Retardation of inflammation has resulted when combined administration of *A. indica* plant extract and venom Ichthyocrinotoxin administered on Carrageenan induced male albino rats (Das *et al.*, 2010). Ethanolic extract of *A. indica* roots at dose 150 mg/kg showed a potent anti-inflammatory effect on compound 48/80 induced paw edema in Wistar male albino rats (Mathew *et al.*, 2011).

Anti-cancer activity

Hepatocellular carcinoma (HCC) in Swiss albino BALB/c mice was induced by carcinogen diethylnitrosamine (DEN) which elevates aspartate transaminases, alanine transaminase, alkaline phosphatase activities. *A. acuminata* root extract significantly attenuated the increased activities of these marker enzymes (Hadem *et al.*, 2014). It was found the root extracts had lowered the levels of tumour necrosis factor-α (TNF-α) levels and nuclear factor kappa-B (NF-κB) activation when analysed the serum and nuclear extracts of DEN induced hepatocellular carcinoma in Albino BALB/c mice. Leaves and stem extracts of this plant also showed chemo-preventive potentiality when tested against six human cancer cell lines (Garg *et al.*, 2007). Fractions of *A. acuminata* root aqueous-methanol extract of 2.5-5mg/ml concentration exhibited the highest inhibition with IC_{50} value of 0.320 mg/ml and induced the effective apoptotic activity determined by MTT assay in HeLa cells (Hadem *et al.*, 2019).

The chloroform leaves extract of *A. indica* showed an inhibitory effect at IC_{50} value at 347 μg/ml compared to the standard anti-cancer therapy drug Taxol when evaluated in human breast cancer cell line (MCF-7 Michigan Cancer Foundation-7) by MTT assay (Subramaniyan *et al.*, 2015).

Anti-diabetic activity

The experimental findings of Karan *et al.* (2012) confirmed the aerial parts of *A. indica* possess significant anti-diabetic properties. A single intravenous injection of aqueous alloxan monohydrate (150 mg/kg) induced diabetes mellitus in Swiss albino mice and Glibenclamide considered as standard drug resulted that after four hours of the administration of chloroform plant extract showed maximum reduction in serum glucose level at the doses of (100, 250, 500, 750 mg/kg, p.o) from 226.3±4.502 to 198.7±2.16 mg/dl, 244.2±3.76 to 206.5±1.871 mg/dl, 414.2±3.869 to 187.2±2.312 and 273±3.742 to 184.7±3.141 mg/dl. Methanolic extracts of *A. indica* roots at doses 100, 200 and 400 mg/kg showed anti-hyperglycemic effect on alloxan induced diabetic mellitus in Sprague Dawley rats and compared with the oral hypoglycemic agent glibenclamide (10 mg/kg). The effect of crude extract on blood glucose levels was measured at various time intervals of 0, 1, 2, 4, 6 and 8 hours. The dose of 400 mg/kg of the crude extract produced a significant maximum fall of 28.94 ± 2.8 on the blood glucose levels of diabetic rats after 6 hours of the treatment compared with disease control group (Goverdhan *et al.*, 2008).

Anti-fertility activity

The anti-fertility activity was evaluated by determining the anti-implantation and early abortifacient activity of ethanolic extract of *A. acuminata* leaves in Wistar rats of either sex orally at the doses of 100 and 200 mg/kg considering 1% Tween 80 as control drug showed significant (100%) antifertility activity on 200 mg/kg in female rats by a significant reduction in the number of corpora lutea and increase in the number of

resorptions (Balaji *et al.*, 2004). The post-coital administration of *A. indica* ethanolic root extract decreased fertility in both Wistar rats and hamsters (Che *et al.*, 1984).

Anti-venom activity

Screening of *A. indica* plant extract against snake (*Daboia russelli)* venom (Meenatchisundaram *et al.*, 2009) and scorpion (*Mesobuthus tamulus)* venom (Attarde and Apte, 2013) showed potent venom neutralizing capacity. 0.11mg of plant extracts were able to completely inhibit PLA2 dependent haemolysis of sheep RBC's induced by *D. russelli* venom and 4 mg of plant extracts were able to completely inhibit PLA2 dependent haemolysis of mice RBC's induced by red scorpion venom in dose dependent manner. The plant extract of *A. indica* is effective in neutralization of lethal venom effects of 2LD50 of *D. russelli* venom and LD99 of *M. tamulus* (red scorpion) venom. Additionally, the pro-coagulant activity showed 1.6 mg and 1 mg of plant extracts were able to completely neutralize coagulant activity in *D. russelli* venom and red scorpion venom clotted human citrated plasma. The modified plaque assay was used to test the fibrinolytic activity, showed 0.11 mg of plant extract was able to completely inhibit fibrinolytic activity (ED50 of 0.5 mg) induced by *D. russelli* venom. The popular bioactive compound of this plant such as Aristolochic acid, Sesquiterpenes, Aristololide works in the modification of the actions of proteins and enzymes which are responsible for the anti-scorpion venom property.

Anti-diarrhoeal activity

The anti-diarrhoeal activity of *A. indica* ethanol and aqueous root extract tested in castor oil-induced diarrhoea male Swiss albino mice resulted the inhibition of 72.38% and 61.94% at a higher dose level 400 mg/kg as compared with diphenoxylate HCl. A delay of the intestinal transit in charcoal meal-induced mice was recorded at the doses of 200 mg/kg and 400 mg/kg of plant extract confirmed the significant result in charcoal induced gastrointestinal motility test (Dharmalingama *et al.*, 2014).

Anti-pruritic activity

Compound 48/80 induced scratched behaviour model was used to evaluate the scratching response of *A. indica* root. The ethanolic plant extract at the dose of 150 mg/kg showed significant effect and decreased the scratching incidence (Mathew *et al.*, 2011). The wound healing potency *A. saccata* leaf extract was studied by Bolla *et al.* (2019). *In vitro* cell-based scratch assay in L929 cells resulted after 48 hours of treatment with 125 µg/mL of plant extract closed the gap created by the scratch by 93.525%. The extracellular matrix (ECM) factor, collagen type-1 might be enhanced by the plant extract which initiated the migration of fibroblasts (Bolla *et al.*, 2019).

Anti-feedant activity

Antifeedant activity studied by Baskar *et al.* (2011) reported the leaf extract of *A. acuminata* was more toxic than the root extract. Maximum anti-feedant activities of 56.06% and 49.86% were recorded on ethyl acetate and hexane leaf extracts of *A. acuminata* at 5.0% concentration against *Spodoptera litura* using leaf disc no-choice method while the root ethyl acetate extract expressed minimum activity of 31.71%. At the same concentration, the ethyl acetate leaf extract exhibited the highest larvicidal activity (40.66%) and pupicidal activity (68.06%). Significant larval toxicity showed by *A. indica* leaf against *Anopheles stephensi.* The formulation of Aristolochic acid I at concentrations of 1000 ppm reduced the survival of all larval instars (Murugan *et al.*, 2015; Pradeepa *et al.*, 2015).

Toxicology

The aristolochic acids found among the species of *Aristolochia* are famous for nephrotoxicity after the tragic Belgian cohort where the women have taken the weight reducing pills contained Chinese herb, *Stephania tetrandra* was inadvertently replaced by aristolochic acid-containing *A. fangchi* were reported to suffering renal

interstitial fibrosis (Balachandran *et al.*, 2005; Debelle *et al.*, 2008). The nephrotoxic and carcinogenic properties of the compound aristolochic acids have been recognised and can cause permanent kidney injury, renal failure (Han *et al.*, 2019). The toxicological risk on the consumption of drugs made up of *A. indica* depends upon several factors like processing, preparation of drugs and mode of administration (Michl *et al.*, 2013). In the quality control of the herbal recipe Homnawakod, Tripatara *et al.* (2012) demonstrated that one of its formulations e.g., the dried roots of *A. acuminata* were not causing nephrotoxicity in rats even the daily administration for 21 days. The acute toxicity study revealed no cytotoxic effects of *A. acuminata* leaves and root, *A. indica* aerial parts and roots when tested in both Swiss albino mice and Wistar albino rats (Balaji *et al.*, 2004; Battu *et al.*, 2011; Mathew *et al.*, 2011; Karan *et al.*, 2012). Leaves of *A. saccata* exhibited mild toxicity against L929 fibroblast cell line at minimum percentage resulted in the death of only 2.88% of cells (Bolla *et al.*, 2019). Michl *et al.* (2013) also reported the contents of Aristolochic acid is higher in leaves, fruits and young stem than roots and woody stems.

Conservation status of *Aristolochia* at the local level

Mostly the roots of the *Aristolochia* sp. are used for a different form of traditional medicine, which arise a problem, as most plants are uprooted directly from the wild before reaching reproductive maturity. This poses a serious threat and is also elucidated by Kayang (2007). However, effective planning on cultivation and management of *Aristolochia* on a small scale can help address this issue, as well as introduction in the home gardens can solve this problem. They can also be planted as beautiful ornamental. The attractive flowers add aesthetic value to its present traditional utilities.

Conclusions

The plants of the genus *Aristolochia* have always been recognized as plants of high medicinal importance by the people of Northeast India. But in recent years, the genus *Aristolochia* L. has been recognized globally for possessing remarkable medicinal value and is reportedly used by people throughout the Indian sub-continent against various diseases and illnesses such as snake bites, muscular ailments, lung, liver and gastrointestinal disorders etc. Hence, in this present study, we have comprehensively reviewed the traditional knowledge on six species of *Aristolochia* found in the northeastern state of Assam along with the various phytoconstituents present in those species. Also, the various properties viz. antimicrobial, antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-fertility, anti-venom, anti-diarrhoeal, anti-pruritic, antifeedant and toxic activity exhibited by various parts of the plant. In short, this review is designed to provide insight into the necessity of further research of important plant compounds to investigate and develop new drugs. Additionally, more comprehensive reviews regarding the activity of the compounds found in *Aristolochia* will help in further development of using *Aristolochia* as an effective drug. Hopefully, these studies will explore the full potential of *Aristolochia* and optimize its use as a promising herbal medicine, thereby promoting global health.

Authors' Contributions

Conceptualization: DB; Data Curation: DB, PJB; Formal analysis: DB, RS; Writing original draft: PB; Writing-review and editing: UD, TJD.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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