IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2019 Vol.:14, Issue:3
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Phytochemical Analysis and Anthelmintic Activity of Different Extract of *Argyreia nervosa* (Aerial Parts)

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Submission: 22 January 2019
Accepted: 29 January 2019
Published: 28 February 2019

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: *Argyreia nervosa*, Phytochemical, Albendazole, Anthelmintic Activity, *Pheretima posthuma*

ABSTRACT

India has an ancient heritage of traditional medicine derived from plant. Materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. So *Argyreia nervosa* is one of the medicinal plants which show many pharmacologically as well as therapeutically effective for the different purposes for human beings. **Aim**: The aim of the study was to investigate Phytochemical screening of ethyl acetate and methanolic extract of *Argyreia nervosa* and the presence of different secondary metabolites responsible for the therapeutic values of the drug like presence of Alkaloids, Glycosides, Carbohydrate, Tannins – Phenolic compounds, Proteins & Amino acids, Gums & mucilage, flavours & flavonoids, saponins and steroids & sterols etc and also to find out the anthelmintic activity study by *in vitro* test species *Pheretima posthuma* responded towards our plant extracts by showing the sign of paralysis and death finally. **Results**: The different extracts collected are ethyl acetate extract yield 3.57% w/w and methanol extract yield 4.93%w/w. Chemical tests on powdered material showed the presence of carbohydrates, proteins and amino acids, fixed oils, alkaloids, phytosterols and glycosides, saponins, and phenolic compounds. **Conclusion**: Chemical tests on ethyl acetate extract showed the presence of fixed oils, alkaloids, phytosterols and glycosides, flavonoids, tannins and phenolic compounds. The data revealed that the ethyl acetate and methanolic extract (5%) of *Argyreia nervosa* (aerial part) showed potent anthelmintic activity.

INTRODUCTION

The plant kingdom is considered as a potential source of new drugs for Centuries. More than 25% of the pharmaceuticals in use today derived from natural product, so interest in natural products research remains strong. This can be attributed to several factors including therapeutic needs that drive a new drug discovery, biologically activities of naturally occurring secondary metabolites, the utility of bioactive of natural products as biochemical and molecular probes, development of novel techniques to detect biologically active natural products, improved techniques to isolate, purify and characterize the active constituents, sufficient supply of bulk products and success of herbal drugs in the global market place. The process of new drug development is enforced by the success of herbal drugs in pharmaceutical market. *Argyreia nervosa* (Burm.f.) Bojer is a Vine Forb/herb and belongs to family Convolvulaceae is a woody herb Grows throughout India, up to an altitude of 300 m, often cultivated in India. The leaves[1, 2] are simple, opposite, elliptical or ovate, petiolate and is of 7.5-3.0 by 6.3-2.5 cm (sometimes even larger), ovate, acute glabrous above, persistently white-tomentose beneath, base cordate; petioles 5-15 cm. long, white-tomentose. pubescent; lamina 3 to 6 cm long and 1 to 3 cm broad; acute or shortly acuminate; more or less pubescent on both sides, base rounded or cordate, venation reticulate; colour-Green to yellowish green when completely dried; odour-unpleasant; taste-bitter and acrid. The flowers [2] are small bell-shaped yellow colour in axillary and lateral umbel like cymes, pedicels long; Calyx- lobes long, ovate, obtuse, pubescent; Corolla pale yellow campanulate, valvate, corona single, with 5 fleshy Scales. Scales andante to throat of corolla tube between lobes. Ovary glabrous Flowers in subcapitate cymes; peduncles 7.5-15.0 cm long, stout, white-tomentose; bracts large, ovate-lanceolate with a long acumen, thin, veined, pubescent outside, glabrous inside, deciduous the outer sometimes 5 cm. long; pedicels very short often almost 0, white-tomentose. Calyx white-tomentose outside; sepals nearly 1.7 cm long, oblong-elliptic, obtuse. corolla 5-6.3 cm. long, tubular-infundibuliform, the bands silky pubescent outside; tube somewhat inflated, white pubescent outside, rose purple and glabrous inside. Fruit[2] 2.0 cm. in diameter, globose, aciculate. Stem[2] stout, white tomentose. The seeds[3] are found in the pods of dried flowers. These cannot be harvested until the pods are completely dried. There are 3 to 5 seeds commonly 4, per flower. Roots[4] of varying sizes and thickness, thin pieces show somewhat smooth brownish exterior, thick pieces tough and woody, light brown in colour, rough, longitudinally striated, lenticellate and with circular root scars; fracture fibrous; rootlets and branches, thin and somewhat fibrous; odour, nil;

taste, pungent, bitter and astringent.Stem[4] are Glabrous or minutely stellately hairy, flattened at the extremities, fibrous, usually woody at the base.

Leaves

The petroleum ether extract of the leaves yielded 1-tricontanol, epifriedelinol acetate, epifriedelinol and β-sitosterol[1,5,6]. The leaves were found rich in quercetin[7]. Extraction of the leaves with 90% methanol led to the isolation of the flavonoids, quercetin and kaemperol together with the latter's glycoside kaemperol-3-o-l-rhamnopyranoside[8]. Two new flavone glycosides characterized as 7,8,3',4',5'-pentahydroxyflavone5-o-α lrhamnopyranoside and 7,8,3',4',5' pentahydroxyflavone5-o-α-l-glucopyranoside were also reported from leaves[9].

Roots

The hexane extract of the root yielded tetradecanyl palminate, 5,8-oxidotetracosan-10-one[10]. The hexane extract of the root yielded two novel aryl esters characterized as stigmasteryl p-hydroxycinnamate and hexadecanyl phydroxycinnmate along with scopoletin[11].

Seeds

The seeds yielded fatty oil which found to contain the glycerides of palminate, stearic, linoleic, linolenic and oleic acids[12]. The seed oil revealed the presence of myristoleic, myristic, palmitic, linoleic, linolenic, oleic, stearic, nonadecanoic, eicosenoic, eicosanoic, heneicosanoic and behenic acids identified as their corresponding methyl esters through GLC[13]. The ethanolic extract of the seeds revealed the presence of a mixture of three alkaloids, out of which only one was characterized as ergometrin. The other constituents isolated were caffeic acid and ethyl caffeate.[1, 5, 14]. Another study also revealed the presence of ergoline alkaloids in the seeds. The ergolines were indicated to be of clavine type[15]. The free amino acids reported in the seeds were glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, tyrosine, praline and α-amino butyric acid[16]. They contain 0.5-0.9% of ergoline alkaloids, agroclavine, chanoclavine -I, chanoclavine -II, elymoclavine, festuclavine, lysergene, lysergol, isoseto clavine, ergine (0.14%), isoergine(0.19%), ergometrine, ergometrinine, lysergic acid α-hydroxyethylamide and isolysergic acid α-hydroxyethylamide. , pennidavine.[1,5]. Besides the alkaloids, seeds yield a fatty oil(10.68%)[1] .

Fruits

The fruits were reported to contain n-tricontanol, β-sitosterol, p-hydroxycinnamoyloctadecanolate and caffeic acid[17].

Traditional uses

The traditional use of this plant *Argyreia nervosa* is used as Gleet, gonorrhoea, strangury and chronic ulcers. A preparation 'Fortege' made from this plant along with several other ingredients is used for curing sexual disorders in males. Another drug 'Speman' consisting of several ingredients of plant material including this species, is reported to exhibit anabolic-cum androgen-like activity in mice[1],(In stomach complaints, sores on foot, smallpox, syphilis, dysentery and diarrhoea,)[5]. The Seed is mixed with those of *Hygrophila auriculata* (schum.) Heine syn. *H. spinosa* T Anders are used as a tonic. Ergoline is responsible for hallucinogenic. Seeds possess significant hypotensive and spasmolytic activities[1]. The leaves is used as Emollient[1,5], local stimulant[1], rubefacient[1], externally in skin diseases[1,2,5], Vesicant[1], ringworm[1], eczema[1], itch[1], internally to cure boils[1], swelling[1], Maturative[2] and absorptive[2], poultics for wounds[2,5]. Antiphlogistic[5], under surface of the leaf, is irritant and used to hasten maturation and suppuration[2], Upper surface is cooling and supposed to possess healing qualities[1], Gleet[5], gonorrhoea[5], strangury[5] and chronic ulcers[5]. The root is used as Aphrodisiac, diuretic and used in gonorrhoea[1, 2], rheumatism and disease of nervous system[1]. Also used as tonic, a paste of the roots along with those of *Asparagus racemosus* (q.v), *Grewia hirsuta vahl* and *Hemidesmus indicus* R.Br. is used for chronic cough and cold, and inconsequent fever[1], Strangury[2], chronic ulcers[2], in synovitis the powdered root is given with milk[1, 2] and syphilis[1], mixed with vinegar, the sap is rubbed over the body to reduce obesity[2], Alternative and tonic[1], powdered root is soaked seven times during seven days in the juice of the tubers of *Asparagus racemosus* and dried, the resulting powder is given in doses of quarter to half a tola with clarified butter for about a month it improves intellect, strengthens body and prevent effects of age[5]. Root of this plant forms an ingredient of compound powder *Ajmodadi churna* useful in rheumatic affectionshemiplegia[2, 5].

MATERIALS AND METHODS:

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, Wagner's, Dragendorff's, Fehling's A & B, α-naphthol, Ferric chloride, Conc. Sulphuric acid, Pyridine, Sodium nitropruside, Acetic anhydride, were purchased from S.D. Fine Chemical, Mumbai.

The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer. Other chemicals were prepared in the laboratory as, 10 % Lead acetate, 10 % Ammonium hydroxide solution, 10 % Ammonia. And to carry out the anthelmintic study of different extracts, we have taken following chemicals as Saline water (Claris Life sciences Ltd., Ahmedabad). Albendazole (Alkem Ltd.) is used as reference standards.

EXPERIMENTAL WORK

Collection and Authentication

The fresh Aerial part *Argyreia nervosa* was collected from local area in the morning hour during the month of September 2018 of Barpali, (Dist-Bargarh, and Odisha). The plant was authenticated by Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D., P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust, and then it was dried in sunlight for one hour and the stem bark was dried under shade and was powdered by the help of mechanical process. The coarse powder have stored in airtight container for further studies.

Fig-1: Aerial parts of plant *Argyreia nervosa*Fig-2 Powder drug of Aerial Part

Extraction[18]:

The dried powder plant material was extracted with Ethyl acetate, Methanol, by successive cold maceration method with increasing order of their polarity. The powdered drug was extracted for 7 days with each solvent. The extract was then filtered using filter paper and the filtrate so obtained was evaporated in a distillation unit. The results thus obtained from the extraction of *Argyreia nervosa* (Aerial part) are shown in (Table 1).

Table 1: Yield, colour

Extracts	% Yield (w/w)	Colour	Consistency
Ethyl acetate	3.57%	Greenish black	Sticky
Methanol	4.93%	Dark black	Greasy

RESULTS AND DISCUSSION:-

The results of extractive value were showed the methanolic and ethyl acetate extract has 4.93%w/w and 3.57%w/w (Table-1). The Soxhlet extraction was done using the ethyl acetate and methanol respectively and the finding showed methanol has a higher percentage of extract 4.93% w/w. From the finding of extractive value of the extracts has been selected for further studies.

QUALITATIVE PHYTOCHEMICAL SCREENING[13, 14, 15]:

The therapeutic significance of plant drugs depends upon the presence of type of constituent(s). Powder drug and different extracts were screened for different phytoconstituents. The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as Carbohydrates, Protein and Lipids that are utilized as food by human being but also for a multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiological effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition. The following tests may be performed on extracts to detect various phytoconstituents present in them.

DETERMINATION OF BIOLOGICAL (ANTHELMINTIC) ACTIVITY[22, 23]:

The anthelmintic study was done by using one *in-vitro* species adult earthworms *Pheretima posthuma* L.Vaill. Earthworms were collected near the swampy water in our locality. The average size of the roundworm was 5-7 cm; average size of the earthworm was 8-9 cm.

These earthworms were identified and services of veterinary practitioner were utilized to confirm the identity of worms.

The suspensions of various extracts were prepared in 2% gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of similar concentrations of the standard drug albendazole were also prepared in distilled water.

Two ml of each concentration of various extracts of *Argyreia nervosa* aerial part and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 2: Preliminary Phytochemical screening of *Argyreia nervosa*

Test/Reagent used	powdered drug	ethyl acetate extract	Methanol extract
1.TEST FOR CARBOHYDRATES			
Molisch's test	+ve	+ve	+ve
Fehling's Test	+ve	-ve	+ve
Benedict's Test	+ve	-ve	+ve
Barfoed's Test	+ve	-ve	+ve
Tollen's phluroglucinol test	+ve	-ve	+ve
Seliwanoff's test	-ve	-ve	-ve
Test for Starch	-ve	-ve	-ve
2.TEST FOR GUMS & MUCILAGE			
Swelling index	-ve	-ve	-ve
3.TEST FOR PROTEINS & AMINO ACIDS			

Ninhydrin test	-ve	-ve	-ve
Biuret Test	+ve	+ve	+ve
Tannic acid test	-ve	-ve	-ve
Millon's Test	-ve	-ve	+ve
Xanthoprotein Test	+ve	+ve	+ve
4.TEST FOR FIXED OILS & FATS			
Spot Test	+ve	+ve	+ve
5.TEST FOR PHYTOSTEROLS			
Liebermann-Burchard	+ve	+ve	+ve
Salkowski's test	+ve	+ve	+ve
6.TEST FOR GLYCOSIDES			
Baljet's test	+ve	+ve	+ve
Legal's Test	+ve	+ve	+ve
Borntrager's test	-ve	-ve	-ve
Modified Borntrager's	-ve	-ve	-ve
7.TEST FOR SAPONIN			
Foam test	+ve	-ve	-ve
8.TEST FOR FLAVONOIDS			
Shinoda test	+ve	+ve	+ve
Lead acetate test	+ve	+ve	+ve
Fluorescence test	+ve	+ve	+ve
Action of alkali and acid	+ve	+ve	+ve
9.TEST FOR TANNINS AND PHENOLIC COMPOUNDS			
Ferric chloride test	+ve	+ve	+ve
10.TEST FOR ALKALOIDS			
Mayer's test	+ve	+ve	+ve
Dragendorff's test	+ve	+ve	+ve
Wagner's test	+ve	+ve	+ve
Hager's test	+ve	+ve	+ve

+ ve Present, -ve Absent

Table 3: Anthelmintic Effect of *Argyreia nervosa* (aerial part) extracts

Group	Concentration of Extract (%)	Time in minutes (Mean ± SEM)	
		Paralysis	Death
Albendazole	1.0	30.4±0.33	41.0±1.45
	2.5	23.8±1.52	36.5±1.4
	5.0	16.6±0.88	28.6±1.2
Ethyl acetate extract	1.0	302.47±1.4	440.66±1.53
	2.5	327.58±1.57	377±1.46
	5.0	189.61±2.16	316.66±0.91
Methanolic extract	1.0	352.64±1.86	454.66±1.86
	2.5	333±2.07	441.66±0.57
	5.0	197±1.99	355.33±2.07

Results are expressed as mean ± SEM from six observations, *Control worms were alive up to 24 hrs. of observation*, N/A= No Activity shown within 24 hours.

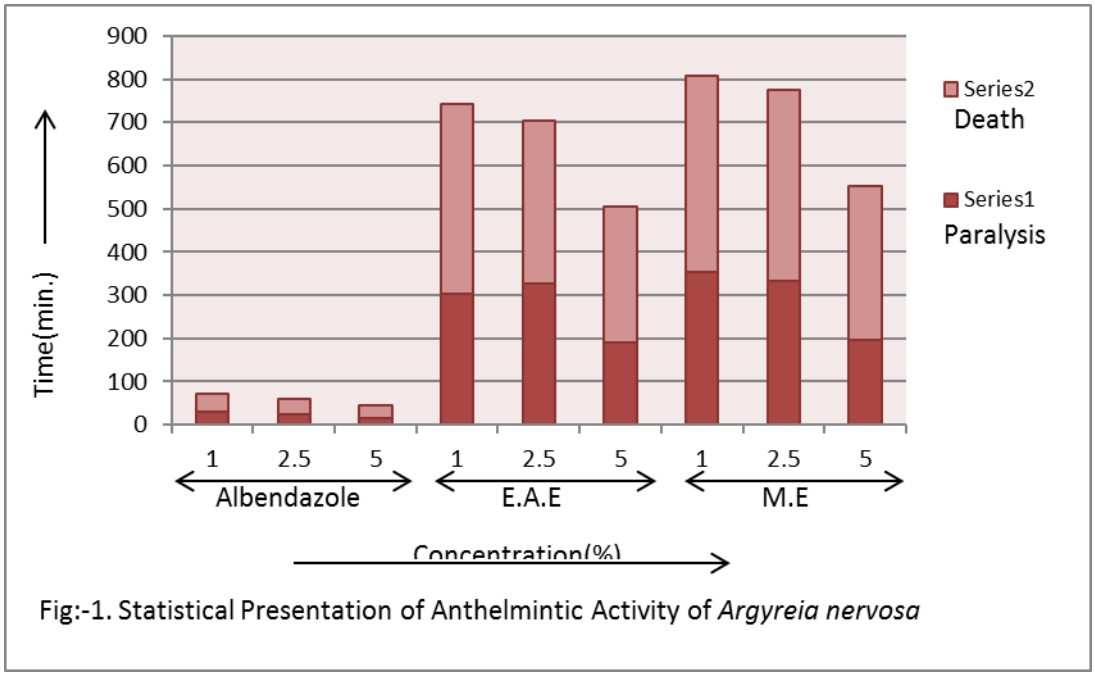

Fig:-1. Statistical Presentation of Anthelmintic Activity of *Argyreia nervosa*

RESULTS AND DISCUSSION:

The finding of phytochemical detection (as was shown in Table 2). Qualitative Phytochemical analysis reports for presence of phytoconstituents in *Argyreia nervosa* are Glycosides, Carbohydrate, Phenolic compounds, Flavonoids and Saponins and many more as stated above. The dried powder plant material was extracted with ethyl acetate, methanol, by successive cold maceration method with increasing order of their polarity. The powdered drug was extracted for 7 days with each solvent. The extract was then filtered using filter paper and the filtrate so obtained was evaporated in a distillation unit. The extracts collected are ethyl acetate extract yield 3.57%w/w and methanol extract yield 4.93%w/w(as shown in Table 2) and observed with naked eyes as well as under U.V light reveal that colour of ethyl acetate extract is greenish black in daylight and brown in U.V light having sticky consistency, colour of methanol extract is dark black in daylight and dark brown in U.V light having greasy consistency. Chemical tests on powdered material showed the presence of carbohydrates, proteins and amino acids, fixed oils, alkaloids, phytosterols and glycosides, saponins, flavonoids, tannins and phenolic compounds, Chemical tests on ethyl acetate extract showed the presence of fixed oils, alkaloids, phytosterols and glycosides, flavonoids, tannins and phenolic compounds, Chemical tests on powdered material showed the presence of carbohydrates, proteins and amino acids, fixed oils, alkaloids, phytosterols and glycosides, flavonoids, tannins and phenolic compounds. The results (as shown in Table 3) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the ethyl acetate and methanolic extract (5%) of *Argyreia nervosa* (aerial part) showed potent anthelmintic activity. The results were compared with the standard drug, Albendazole. The present study justifies the folklore claims of the anthelmintic activity of *Argyreia nervosa* (aerial part)**.** Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

ACKNOWLEDGEMENTS

The authors sincerely thanks to the Principal, Director and Management of The Pharmaceutical College, Barpali, Bargarh for providing all the facilities to carry out the study.

CONFLICT OF INTEREST: None

SOURCE OF FUNDING: Nil

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