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Pharmacognostical and phytochemical study of Arjuna *(Terminalia arjuna Roxb)*

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ABSTRACT

Arjuna is one among the *hridyadravya* used in many aliments including cardio vascular diseases since vedic period.Though *arjuna* is botanically identified as Terminalia arjuna belongs to combrataceae family,Other species of Terminalia are being sold indiscriminately under the name of *Arjuna*. Pharmacognostical and phytochemical studies help in identification of a drug in a systematical manner. Present work aims to validate genuinity of Arjuna by phytochemical and pharmacognostical studies. Organoleptic, macroscopic, microscopic, physicochemical and fluorescence features were studied along with their phytochemical features using methods given in Indian Ayurvedic Pharmacopoeia. TLC and HPTLC studies were carried out by using suitable solvents and results were discussed in detail.

KEY WORDS *Arjuna, Hridya*,Cardio vascular disease,Pharmacognostical, Phytochemical.

Introduction

Medicinal plants has assumed greater importance in the present era. They are using tremendously against many diseases of mankind due to their high potentiality**.** Arjuna botanically identified as *Terminalia arjuna roxb* belongs to Combrataceae family. The tree is common throughout the greater parts of Indian peninsula along Rivers, Streams, and dry water courses. Found in sub Himalayan tract, Orissa, West Bengal, Punjab, Madhya Pradesh, Tamil Nadu, Bengal, Bihar and in Karnataka[1,2].*Charaka* delineated *Arjuna* in the *udardaprashamana* group. *Bruhathrayis* have indicated it for *Raktapitta, Arsha*, and *Kusta* etc. It is

vrnda, Chakrapani and sodhala who have high lighted the role of *Arjuna* in *Hrudroga*[3,4]. Sushrutha mentioned *Arjuna* and *kakubha* separately. Dalhana in this context mentioned that *kakubha* is a shrub with aromatic root and it may be *Artagula*. Vagbhata mentioned it by synonyms, *Dhananjaya* and *shwethavaha*[3].The Bark is useful externally in Wounds, Fracture, Ulcers, Diseases of Heart, Anaemia, Excessive perspiration, Asthma, Tumors, Leucoderma, Dysentery, Diarrhea. Etc. *Arjuna* being *Kashayarasa,Katuvipaka* and *Sheetaveerya* act as *Kaphavata shamaka*[5,6,7] and supposed to have chemical constituents like glycosides,tannins ,saponins ,plant acids like ellagic acid [8,9]etc. which have shown their effect in many disorders including cardiac aliments. The dried bark of the plant Terminalia tomentosa N & A and Terminalia Alata Heyne ex Roth are most common adulterants using under the name of Arjuna. More than 15 other species of Terminalia are being sold indiscriminately under the name of Arjuna like T. bialata steud T. Bellarica Roxb T. Manni King [9,10,11]etc. Hence an attempt has been made to know the genuinity of *Arjuna* bark collected from market through pharmacognostical and phytochemical studies.

Material and Methods

The present work has been carried out under following headings:

- Macroscopical study
- Microscopical study
- Determination of Physical constants
- Phytochemical analysis
- TLC And HPTLC studies

- Botanically identified dried stem bark of Arjuna was collected from sanjeevini pharmacy, kengeri Bangalore, which had its characteristic features.
- Methonolic Extraction was carried out by Soxhlation technique.
- Fluorescence Analysis by Using UV Chamber was carried out with the crude powdered drug exposed as such and exposed with water, ethanol, and with dil. Hcl.

- Organoleptic study was carried out for the parameters – Colour, Odour, Texture, and Taste.
- Microscopic Study of Arjuna bark was carried out by using 1% safranin stain.
- Physical constants like determination of Foreign matter, Moisture content, Ash content, Acid insoluble ash, Alcohol soluble extractive value,Watersoluble extractive value,P^Hvalue are carried out according to standard procedures.
- Phyto chemical analysis was carried out by using standard reagents.
- TLC wascarriedout by using Benzene:Ethanol and Benzene:Ethylacetate as solvents for mobile phase.
- HPTLC was carriedout by using Toluene:Ethylacetate:Formicacid: Methanol as solvent for mobile phase.

Observations and results

Organoleptic obseservation of arjuna bark

Sl. No	**Observations**	***Arjuna* bark**
1.	*Shabdha*	Not easily breakable
2.	*Rupa*	Reddish Brown
3.	*Rasa*	Astringent
4.	*Gandha*	Characteristic of astringent.
5.	Identifying features	Flat-channelled, Reddish brown colour with mealy white coating on external surface.

Observations during preparation of methanolic extracts

Sl. No	**Observations**	***Arjuna* bark Extract**
1.	Colour	Reddish
2.	Consistency	Solid

3.	Nature	Crystalline powder
4.	Odour	Characteristic odour
5	Taste	Astringent
6.	Colour of prepared solution	Reddish Brown

UV Analysis: The colour changes on exposure to UV rays with different reagents

Sl. No.	**Samplewith reagent**	***Arjuna***		
		Visible rays	UV 365μm	UV 254μm
1	Powder as such	Reddish Brown	Light greyish Brown	Dark brown
2	Powder with water	Reddish Brown	Brown	Blackish Brown
3	Powder with Dil.Hcl	Reddish Brown	Blackish Brown	Reddish Brown
4	Powder with ethanol	Brown	Brown	Blackish Brown

UV analysis of the drug *Arjuna*

Macroscopical Characters bark:

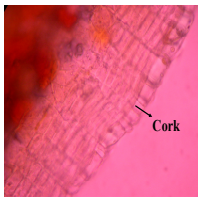
- Market sample occured in 10cms × 9cms
- Bark flat, slightly channelled, 02 – 25cms thick
- Outer surface was smooth
- Inner surface was fibrous and pinkish
- Fracture- it was not easily breakable.

Microscopical characters:

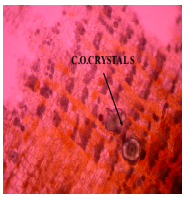
- T.S of the bark (mature) shows cork consisting of 9 – 10 layers tangentially elongated cells, few outer layers filled with Brown content of **tannin.**
- Cork cambium single layered, secondary cortex many layered made up of thin walled closely arranged **parenchymatous cells**.
- Medullary ray single layered (uniseriate) traverses up to outer bark region. Secondary cortex is filled with abundant rosette **calcium oxalate** crystals and simple **starch grains**.
- Secondary phloem is wide and some of the cells show rosette **calcium**

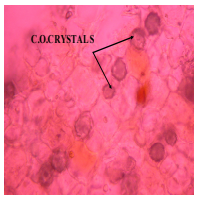
oxalate crystals. **Sclerenchymatousfibers** alternate with the phloem cells and some of the phloem cells shows reddish to orange red **tannin** content.

Macroscopy

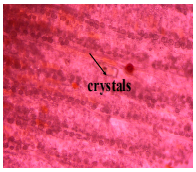


Microscopy

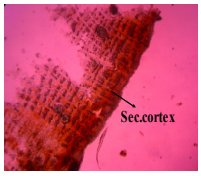




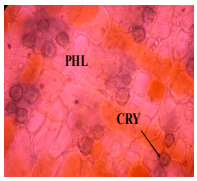
Calcium oxalate crystals (10Xx 40X)

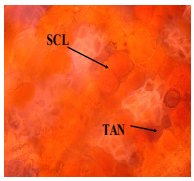


Abundant crystals in sec.cortex

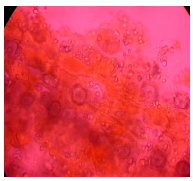


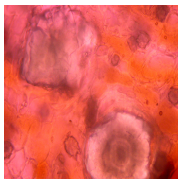
T.S.of the bark showing sec.cortex



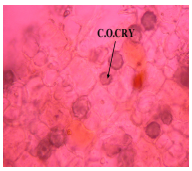


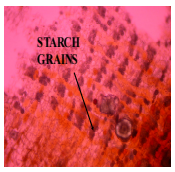
showing sclerenchyma , crystals and tannin content





Abundant calcium oxalate crystals and starch grains in secondary cortex region





Portion showing phloem(10Xx40X) calcium oxalate crystals and starch grains

Physico-chemical analysis:

Sl. No	Test	Permissible limit for *Arjuna*[12]	Obtained percentage for *Arjuna*
1	Loss on drying at 110°C	-	5.28%
2	Total ash	Not more than 25%	18. 79%

3	Acid insoluble ash	Not more than 1%	0.45%
4	Water soluble extractive	Not less than 20%	25.312%
5	Alcohol soluble extractive	Not less than 20%	22.80%
6	PH value	-	6.5
7.	Foreign matter	Not more than 2%	0.5%

Phytochemical analysis:

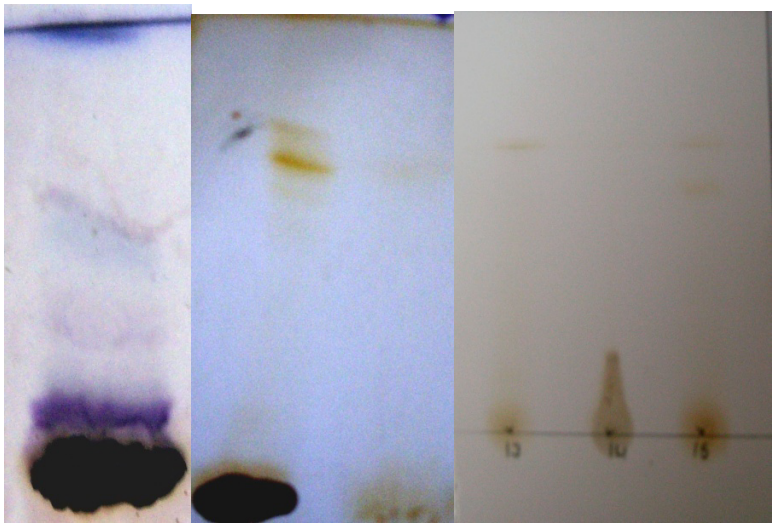
PHYTO CHEMICAL	TESTS	PROCEDURE	OBSERVATION	RESULTS
Alkaloids	Dragendroffs test	The extract was dissolved in dilute sulphuric acid, and to this acidic solution, Dragendroffs reagent was added.	Reddish-brown precipitate indicates presence of alkaloids	Absent
	Mayer's test	The extract was dissolved in dilute sulphuric acid, and to this acidic solution, Mayer's reagent was added	Yellow precipitate indicates the positive test.	Absent
Glycosides	Modified Borntragers test	To the extract 5ml of 5% ferric chloride solution and 5ml of dilute HCL were added and boiled on water bath. After cooling, benzene or any organic solvent was added and shaken well. The banzen layer was separated and	Pinkish red colour of Ammonical layer indicates the presence of glycosides.	Present

		equal volume of dilute ammonia was added.		
Triterpenoids	Salkowski test	A Few drops of concentrated sulphuric acid were added to the chloroform solution, shaken well and allowed to stand.	Golden yellow colour of lower layer indicates the presence.	Present
Saponins	Foam Test	To the filtrate, Few ml of water was added and shaken well.	Froth is produced.	Present
	Froath test	To the filtrate, few drops of sodium bicarbonate solution were added. the mixture was shaken vigorously and left for few minutes.	Honeycomb like froth was produced.	Present
Tannin	Ferric chloride test	To the alcoholic extract dissolved in water +few drops of 5% $FeCl_3$ solution.	Blue, black Brownish or green precipitate indicates presence.	Present
Steroids	Salkowski reaction	2ml chloroform solution+1ml Con. H_2SO_4 added from side of test tube	Red colour indicates the presence.	Present
Flavonoids	Ferric chloride test	2ml of $FeCl_3$+2 drops of Alc.Ext.	Blackish red indicates the presence.	Present
Carbo hydrates	a. Benedicts test	0.5ml Alc.Ext+ 5 ml of benedicts solution, boil for	Reddish brown ppt indicates presence	Present

		5min		
	b. Fehlings test	2ml of Alc. Ext+ 1 ml of fehlings sol. (Mixture of sol. A and B), boil for few min.	Red ppt. Indicates the presence.	Present
Proteins	Biuret's test	To the filtrate, Biuret"s reagent was added.	Violet colour indicates the presence	Present

TLC findings of the drug *Arjuna*

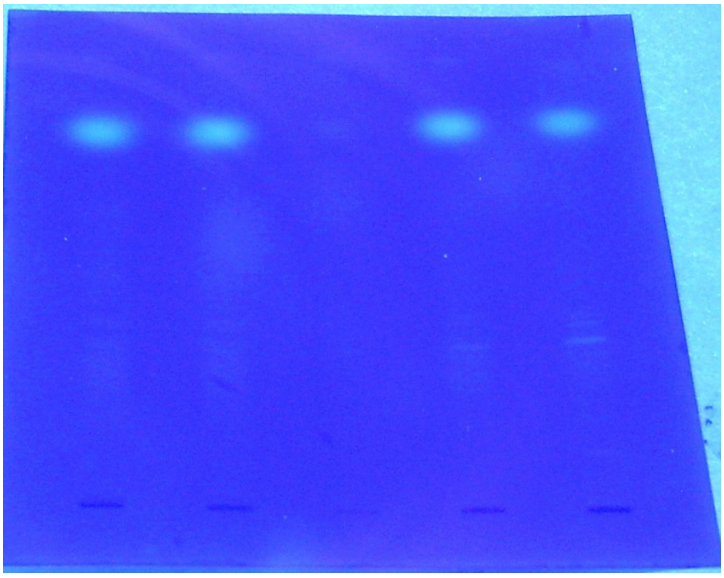
TLC findings of the drug *Arjuna* in different dosage forms				
Extract used	**Phytoconstituents**	**Mobile phase**	**Solvent front**	**Rf values**
Alcoholic extract	Glycosides	Benzene Ethanol(7:3)	4 cms	0.9
	Flavonoids	Benzen, Ethylacetate(4:1)	6.7 cms	0.5



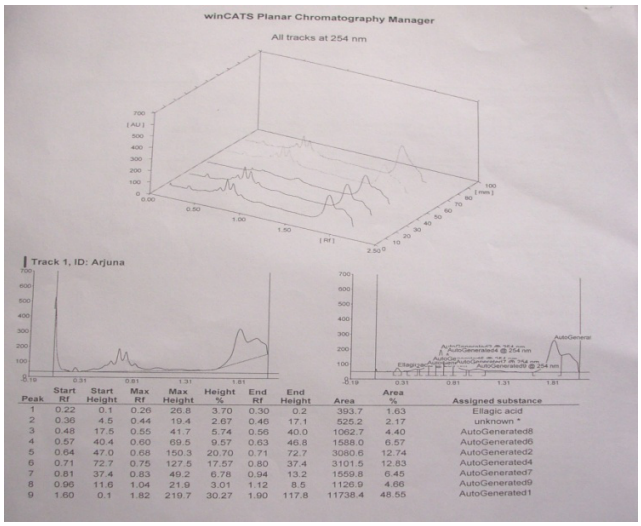
Glycoside-extract Flavonoid-extract

HPTLC findings of the drug*Arjuna*

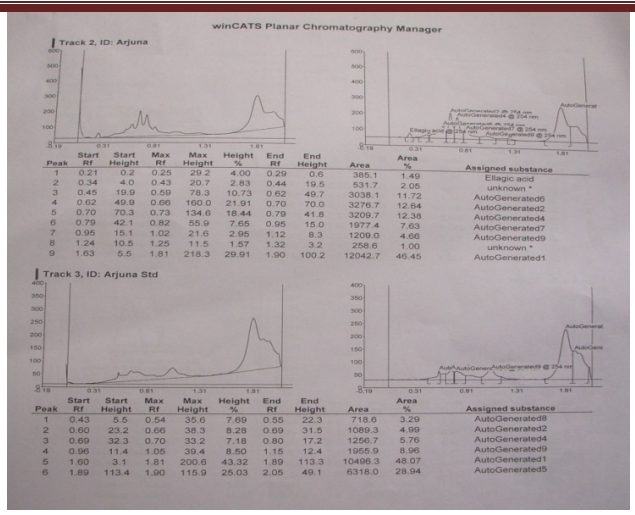
DRUG	CONSTITUENT	SOLVENT	Rf VALUE
Arjuna	Ellagic acid	Toluene:ethylacetate:formic acid: methanol(6:3:0.1:1)	0.26



ARJUNA



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.22	0.1	0.26	26.8	3.70	0.30	0.2	393.7	1.63	Ellagic acid
2	0.36	4.5	0.44	19.4	2.67	0.46	17.1	525.2	2.17	unknown *
3	0.48	17.5	0.55	41.7	5.74	0.56	40.0	1062.7	4.40	AutoGenerated8
4	0.57	40.4	0.60	69.5	9.57	0.63	46.8	1588.0	6.57	AutoGenerated6
5	0.64	47.0	0.68	150.3	20.70	0.71	72.7	3080.6	12.74	AutoGenerated2
6	0.71	72.7	0.75	127.5	17.57	0.80	37.4	3101.5	12.83	AutoGenerated4
7	0.81	37.4	0.83	49.2	6.78	0.94	13.2	1559.8	6.45	AutoGenerated7
8	0.96	11.6	1.04	21.9	3.01	1.12	8.5	1126.9	4.66	AutoGenerated9
9	1.60	0.1	1.82	219.7	30.27	1.90	117.8	11738.4	48.55	AutoGenerated1



DISCUSSION

Ayurveda gives due importance to second *chathushpada* i.e. *dravya* which is the radicle of ayurvedic pharmacology.

Dravya is the base for multifarious pharmacological activities.

Pharmacognostical and phytochemical studies will help in evaluating the drug for its genuinity.

Arjuna belongs to combrataceae family is used extensively in therapeutics specially in *hridroga.*

In organoleptic observation, Arjuna was reddish brown color, probably indicates the presence of tannin content.

The UV analysis carried out showed Brown, Blackish brown and brown colour with water,dilute HCL and Ethanol at 365μm and blackish brown, Reddish brown and Blackish brown at 254μm with same reagents confirms the genuinity of *Arjuna.*

T.S of the bark (mature) showed cork consisting of 9 – 10 layers tangentially elongated cells, few outer layers filled with Brown content of **tannin.**

Secondary cortex showed abundant rosette **calcium oxalate** crystals and simple **starch grains**, which serves as an identification characters.

Physic chemical analysis shows Ash value 18.79%,Acid insoluble ash 0.45%,Water soluble extractive

value 25.31%, and Alcohol soluble extractive value 22.8% which were in the permissible limits.

18.79% ash value in *Arjuna* indicates presence of inorganic constituents like calcium, iron, carbonates, chlorides, sulphates.

Phytochemical analysis carried out for Arjuna showed the presence of tannins, steroids, flavonoids, carbohydrates, saponins, glycosides and proteins.

The TLC study showed the presence of glycosides with Rf value 0.9 and flavonoids with Rf value 0.5. which are having cardio protective activity, and increase capillary permeability.

Flavonoids basically contain free hydroxyl groups and exert a good physiological effect on capillaries and they have coronary dilatory effect on heart. Glycosides, especially present in Arjuna can probably improve the function of heart through atrio ventricular conduction and vagal tone.

The HPTLC study carried out showed the presence of Ellagic acid (hydrolysable tannin), with Rf value 0.26.

Tannins usually having hypotensive action with vaso dilatation and decreases heart rate and probably prevent putrefaction and act as cardio tonic. Hydrolysable tannins basically enhance the healing process.

Conclusion

In the present work, macro and microscopic studies revealed the indentification characters of the drug *Arjuna.*

Physico chemical study carried out for Ash value and Extractive values showed the values in permissible limits which indicates the drug is genuine.

Phytochemical study showed the presence of Glycosides, Flavonoids, Tannins etc which are reconfirmed by TLC.

HPTLC study showed the presence of Ellagic acid with the Rf value 0.26. which proves the genuinity of the drug.

A small attempt has been made to know the genuinity of Arjuna

through this work, further studies can be made by comparing the different samples collected from different source.

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