World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

PHAMACOGNOSTIC AND PHYSICOCHEMICAL EVALUATION OF ASHWAGANDHADI COMPOUND- A POLYHERBAL AYURVEDIC FORMULATION

[1]*Dr. Nilesh M. Ingle, [2]Prof. P. K. Dash and [3]Dr. Ashwini N. Ingle

[1]PhD Scholar, P.G. Department of Kaumarbhritya, YAC, PhD Centre Kodoli.(Mh).
[2]Prof. & HOD, P.G.Deptt. of Kaumarbhritya, YAC, PhD Centre Kodoli.(Mh).
[3]Asso. Prof. Department of Kayachikitsa, Patanjali Ayurved College, Haridwar.

Corresponding Author: Dr. Nilesh M. Ingle
PhD Scholar, P.G. Department of Kaumarbhritya, YAC, PhD Centre Kodoli.(Mh)

Article Received on 26/05/2022 **Article Revised on 16/06/2022** **Article Accepted on 06/07/2022**

ABSTRACT

Ashwagandhadi Compound is a polyherbal Ayurvedic formulation which is prepared in granule form and has *Ashwagandha* (Withania somnifera), *Brahmi* (Bacopa monnieri), *Shankhapushpi* (Convolvulus pluricaulis), *Vacha (Acorus calamus*) and *Jatamansi* (Nardostachys jatamansi). The present study deals with evaluation of Phamacognostic and Physicochemical Evaluation of *Ashwagandhadi* Compound. After Pharmacognostic evaluation the total ash value found to be 2%, acid-insoluble ash value 0.5%,water-soluble extract 60% w/w, alcohol-soluble extract 40% w/w and loss on drying weight difference after 3hours was found to be 0.5gm. High performance thin layer chromatography were carried out after organizing appropriate solvent system in which maximum five spots were distinguished and few of the Rf values were identical in the alcoholic extract. It is evident from the physiochemical evaluation that present formulation contains Carbohydrates, reducing sugars, Flavonoids, Alkaloids, Tannins and Glycosides.

KEYWORDS: High performance thin layer chromatography, Pharmacognosy, *Ashwagandhadi* Compound.

INTRODUCTION

Herbal medicines possess therapeutically strong background to meet health needs of human kind. Herbal medicines; either single or in combination contains many active principles which can be credited for their efficacy. But due to high variability of chemical constituents of herbal drugs; creates a challenge in establishing the specific quality control standards of finished products.

In present study *Ashwagandhadi* compound,[1] a polyherbal compound was converted to granules by *rasakriya* method to make it palatable. In addition to that granules help in fixing the dose, easy to administer, and also increases the shelf life.[2] As no standard finger print is available for this compound, an attempt has been made to evaluate pharmacognostical features and Standardization of *Ashwagandhadi* (AG) compound parameters including organoleptic characters, physicochemical analysis and chromatographic pattern.

Aims & Objective

1. Pharmacognostical study of powdered drugs – *Ashwagandhadi* compound
2. Physico-chemical analysis of *Ashwagandhadi* compound

MATERIAL AND METHODS

1. Collection and authentication of raw drugs

The formulation composition of Ashwagandha (AG) Compound was obtained from Rasashala, Vidarbha Ayurved Mahavidyalaya, Amravati Maharashtra. The API standards were used for pharmacognostical authentication of *Ashwagandha, Brahmi, Shankhapushpi, Vacha, Jatamansi*based on the morphological features, organoleptic characters and powder microscopy of individual drugs.

2. Method of preparation of Ashwagandhadi Compound

General method for preparation of *khandapak* was followed for the preparation of *Ashwagandhadi* compound.[3] Raw materials in dried form (Table 1) were taken in equal quantity were crushed to prepare coarse powder. As per the classical method of decoction preparation, decoction of *Ashwagandhadi* Compound was made in ratio of 1:16:1/8. One part raw drugs as to sixteen part of water boiled upto desirable quantity of one eighth part. Continuous stirring was carried to facilitate the evaporation and avoid any deterioration due to burning of materials. Prepared decoction was filtered through single folded cotton cloth and collected into a separate vessel. After filtration sugar was added to that

decoction in equal proportion of coarse powder. Subsequently the decoction was boiled over slow fire to obtain a semisolid consistency. With evaporation of water, the viscosity of the decoction increased resulting in solid mass (*ghan*). At last solid mass was pass through a sieve no.8 to prepare granules.

Table 1: Ingredients of *Ashwagandhadi* Compound.

S.N.	Name	Latin Name	Parts used	Ratio
1.	*Ashwagandha*[4]	Withania somnifera	Root	1 part
2.	*Brahmi* [5]	Bacopa monnieri	Whole plant	1 part
3.	*Shankhapushpi*[6]	Convolvulus pluricaulis	Whole plant	1 part
4.	Vacha[7]	Acorus calamus	Root	1 part
5.	*Jatamansi*[8]	Nardostachysjatamansi	Rhizome	1 part

3. Pharmacognostical evaluation

Pharmacognostical analysis of AG Compound included organoleptic characters and microscopic studies. Raw drugs were coded as Brahmi (BHM 50), Ashwagandha (AGM 50), Jatamansi (JMM 50), Shankhapushpi (SKM 50) and Vacha (VM 50).

Fig. 1: Showing powders of individual drugs of AG Compound.

3.1 Table 2: Organoleptic Evaluation of Individual drugs.

Sample code	BHM 50	AGM 50	JMM 50	SKP 50	VM 50
Colour	Greyish	Creamy White	Dark Brown	Creamy	Brown
Odour	Characteristic	Strong haracteristic	Pungent And Characteristic	Characteristic	Aromatic
Taste	Bitter	Bitter	Pungent	Bitter	Bitter

3.2 Table 3: Ph of Ingredients.

Sr. no.	Sample code	pH
1.	BHM 50	5.5
2.	AGM 50	5
3.	JMM 50	5
4.	SKP 50	5
5.	VM 50	5

3.3 Table no 04 - Loss on Drying (%LOD)

The 2g quantity of the sample is placed in the previously dried weighing bottle. The Drying was carried out in an oven at a temperature 105°C until the constant mass of the substance was obtained.

Results: %LOD=W3-W2/W2-W1*100

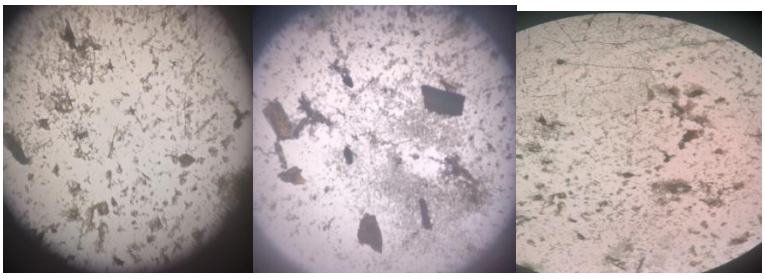
SAMPLE CODE	Wt.of empty Petri plate (W1)	Wt.of empty plate + Sample(W2)	Wt.of plate after drying(W3)	% LOD (% w/w)
BHM 50	39.90 g	40.90 g	40.86 g	5%
AGM 50	44.72 g	45.76 g	45.72 g	1%
JMM 50	44.74 g	45.70 g	40.70 g	8.5%
SKP 50	41.01 g	42.00 g	42.00 g	4%
VM 50	44.62 g	45.58 g	45.58 g	3.84%

3.4 Powder Microscopy

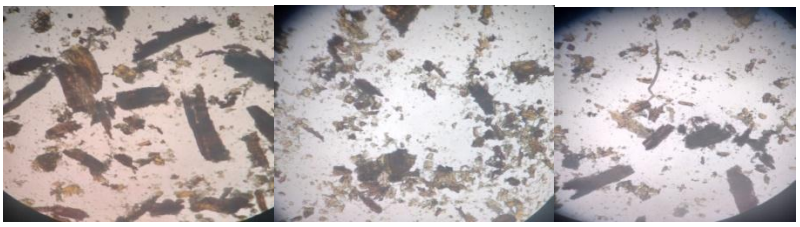
Powder was cleared with clearing agents. Thin layer of powder was spread on glass slide and observed under microscope. To differentiate cells (lignified and non-lignified, starch grains, oil glands) staining reagents were used. Following characters were observed according to plant parts.

Table 05: Common Powder characteristics.

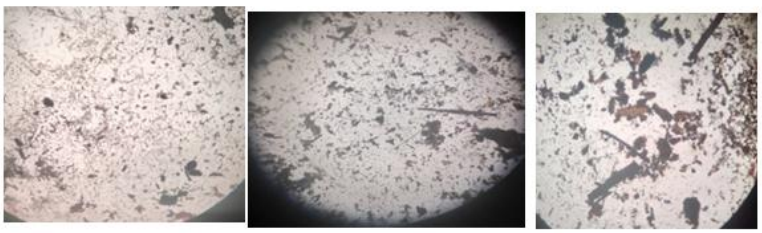
Leaves	Epidermal cells, palisade cells, stomata, trichomes, calcium crystals, starch grains,
Roots/Rhizomes	Cork cell, parenchyma cells, phloem fibers, xylem, calcium crystals, starch grains, stone cells
Bark/wood	Cork cell, parenchyma cells, phloem fibers, xylem, calcium crystals, starch grains, stone cells, pericyclic fibers, sclerides, fibers
Flowers	Epidermal cells, anthers, pollen grains, oil globules, pigments,
Seeds	Endosperm, oil glands, aleurone grains, starch grains, pigment
Fruits	Epidermal cells, pericarp, mesocarp, oil glands (vittae), sclerenchymatous cells



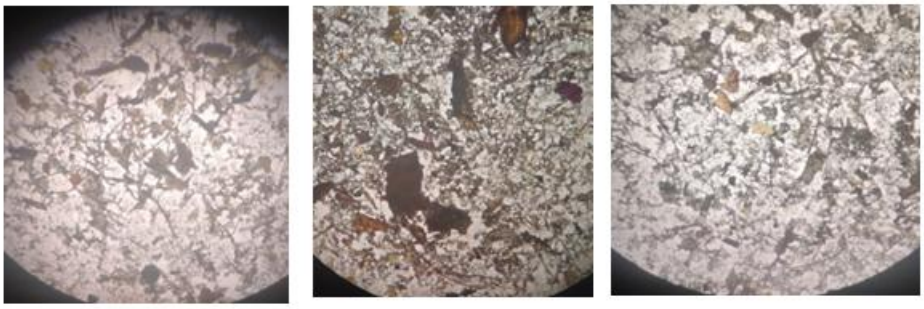
1. Fig no. 02 - BHM 50 : Diagnostic character of Brahmi shows prismatic, cluster crystals of calcium oxalate, starch grains and oil globules scattered as such throughout or embedded in the parenchymatous cells; fragments of longitudinally cut annular and pitted vessels.



2. Fig no. 03 AGM 50: Cork cells, Pitted and scalariform vessels, Tracheids, Scalariform vessel, Parenchyma cell packed with starch grains, Starch granules with stellate hila.



3. Fig no. 04 JMM 50: Powder shows hairs, Medullary rays, and Cells filled with reddish brown contents, fibers, Cork cells in surface view, Xylem vessels with pitted and scalariform secondary wall thickenings and starch grains.



4. Fig no. 05 SKP50: Powder shows group of pitted, spiral and annular vessels, unicellular hairs, pollen grains with thick exine, fibers, epidermal cells with paracytic type of stomata, palisade cells, cork cells.

5. Fig no. 06 VM 50: Powder shows Fiber, Starch grain, Vessel.

Table 06: On the basis of morphological, microscopically and physicochemical studies, given crude drug powders are identified and confirmed as follows.

Sr. No.	Code	*Identified as*	*Part*
1	BHM 50	*Brahmi* (*Bacopa monieri*)	Leaves
2	AGM 50	*Ashwagandha* (*Withaniasomnifera*)	*Root*
3	JMM 50	*Jatamansi* (*Nardostachysjatamasni*)	Roots
4	SKP 50	*Shankhapushpi(Convolvulus pluricaulis)*	*Whole plant*
5	VM 50	*Vacha* (*Acorus calamus*)	Roots

Physicochemical Evaluation Of *Ashwagandhadi* Compound

1. Organoleptic Evaluation

Ashwagandhadi Compound was found brown in Colour with characteristic odour and sweet characteristic taste.

2. Macroscopic Evaluation

Light Brown Colored Granular Powder with crystals of sugar was observed. No foreign particle nor any traces of stones, insects, fungal contamination were seen. Granule was in good condition at the time of analysis.Further evaluation showed presence of Lignified xylem, vascular bundle, Lignified fiber, cuticle with oil globules, starch grains and calcium oxalate crystals with respective tests.

3. Physicochemical Parameters

Total ash value of the sample was observed as 2% whereas Acid insoluble ash value of the sample noted as 0.5%. Alcohol soluble extractive value of the sample came as 40 % and that water soluble extractive value of the sample was 60 %. Loss On Drying w/w was 0.5 g after 3 hours of duration.

4. Pharmaceutical Parameters

Bulk density was 0.64 gm/ml and Tapped density was 0.73 gm/ml. Hausner's ratio found to be 1.14 with Car's Index as 9% and Angle of repose was calculated as 37˚.

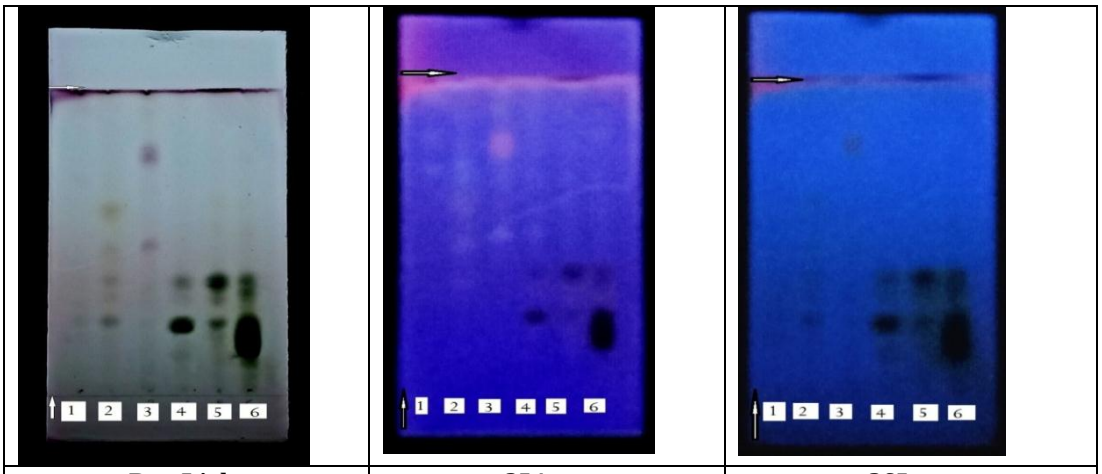
5. Table no. 07 Thin Layer Chromatography Analysis

1. Mobile Phase: Chloroform: Glacial acetic acid: Methanol: Water = 6:3.2:1.2:0.8

2. Stationary Phase: Silica Gel GF254

3. Spraying agent: Anisaldehyde sulphuric acid

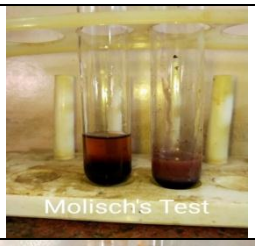
Spot No	Name of Sample	Rf Values of spots detected from starting line up to solvent front
1	Jatamansi	0.22, 0.30, 0.73, 0.89
2	Shankhpushpi	0.23, 0.30, 0.35, 0.44, 0.57, 0.94
3	Brahmi	0.16, 0.23, 0.35, 0.41, 0.52, 0.74, 0.89
4	Ashwagandha	0.12, 0.23, 0.30, 0.37, 0.74
5	Vacha	0.12, 0.16, 0.23, 0.30, 0.35, 0.74, 0.94
	Granules	**0.06,0.11, 0.19, 0.23, 0.32, 0.51, 0.62,0.74, 0.25, 0.94**

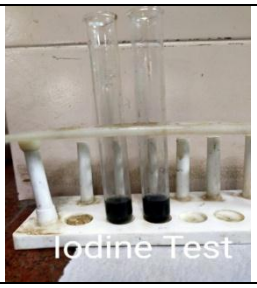
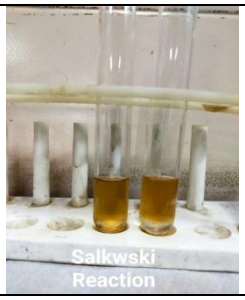
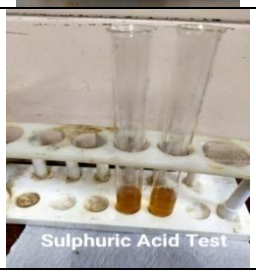
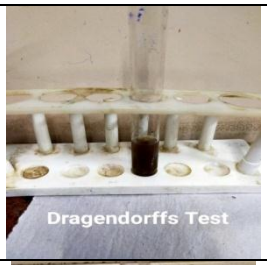
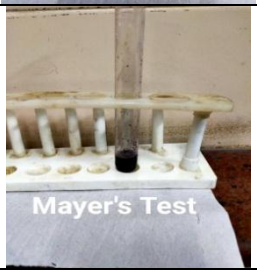


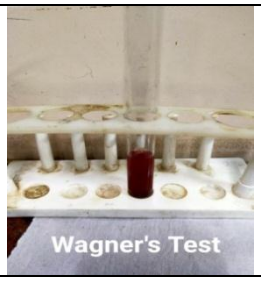
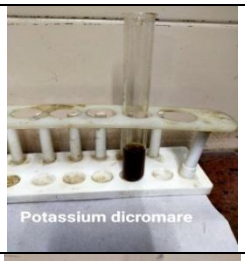
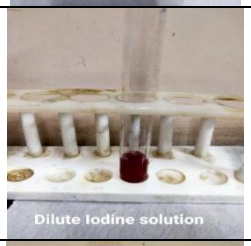
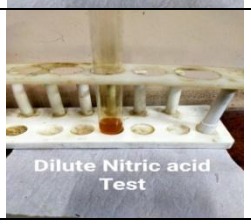
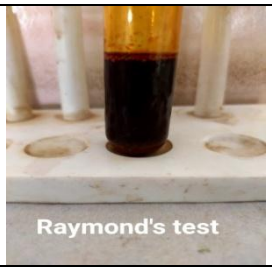
Day Light	254 nm	365 nm

Fig. 07: Thin Layer Chromatography of AG compound

Table 08: Preliminary Phytochemical Screening of Ashwagandhadi Compound.

Sr.No	Chemical Test	Observation	Seen Parameter	Result
1.	**TEST FOR CARBOHYDREDS**			
a)	Molisch's test		Produce violet ring at the junction	Carbohydrate present
b)	Fehling test		Brick red ppt observed	Carbohydrate present
c)	Benedict's test		Green to yellow color present	Reducing sugar present
d)	Test for Monosaccharide Barford's test		Red ppt observed	Monosaccharides present
e)	Aniline acetate test	-	Filter paper turn green	Pentose sugar absent
f)	Tollen's phloroglucinol	-	Yellow to red color observed	Hexose sugar present

	test			
g)	Iodine test		Blue color appeared then disappeared	Non reducing polysaccharide sugar is present.
3.	**TEST FOR STEROIDS**			
a)	Salkowski reaction		Not found red or green ppt	Steroids absent
4.	**TEST FOR FLAVONOIDSS**			
a)	Shinoda test		Found orange color	Flavonoids present
b)	Sulphuric acid test		Deep yellow color found	Flavonoids present
5.	**TEST FOR ALKALOIDS**			
a)	Dandruff's test		Brown ppt observed	Alkaloids are present
b)	Mayer's test		Ppt observed	Alkaloids are present

d)	Wagner's test		Red ppt observed	Alkaloids are present
6.	**TEST FOR TANNINS**			
a)	Potassium Dichromate test		Red ppt	Tannins present
b)	Dilute iodine solution test		Red solution	Tannins present
c)	Dilute HNO_3 test		Red to yellow color	Tannins present
7.	**TEST FOR GLYCOSIDES**			
a)	Raymond's test		Violet color present	Glycosides present

OBSERVATION AND RESULT

A) Table no. 09- On the basis of morphological, microscopically & physicochemical studies, given crude drug powders are identified and confirmed as

Sr. No.	Code	***Identified as:***	***Part***
1	BHM 50	Brahmi (*Bacopa monieri*)	Leaves
2	AGM 50	Ashwagandha (*Withaniasomnifera*)	*Root*
3	JMM 50	Jatamansi (*Nardostachysjatamasni*)	Roots
4	SKP 50	*Shankhapushpi(Convolvulus pluricaulis)*	*Whole plant*
5	VM 50	Vacha (*Acorus calamus*)	Roots

Table 10: On the basis of various parameters and specific tests and HPTLC following observations were noted for the Ashwagandhadi Compound.

S.N.	Parameters	Observation/Result	Remark
1	**Organoleptic Evaluation of Granules**	Light brown colored powder with characteristic odor and sweet taste	Pass the test
2	**Macroscopic Evaluation of Granules**	Sugar crystals observed, No foreign particles, stones, insects are present in powder. No fungal contamination observed	Powder is free of contamination
3	**Microscopic Evaluation of Powder of Granules**	Lignified xylem, vascular bundle, Lignified fiber, cuticle with oil globules, starch grains and calcium oxalate crystals are present	Pass the test
4	**Physicochemical Parameters**		
	Total Ash Value	2 %	Pass the test
	Acid- Insoluble Ash Value	0.5%	Pass the test
	Alcohol soluble extractive value	40%	Pass the test
	Water soluble extractive value	60%	Pass the test
	Loss on Drying	Weight difference after 3 hours was found to be 0.5 gm	Pass the test
	Pharmaceutical Parameters		
	Bulk density	0.64 gm/ml	Pass the test
	Tapped density	0.73 gm/ml.	Pass the test
	Car's Index	9%	Excellent flow property
	Hausner's ratio	1.14	Good flow property
	Angle of repose	37˚	Fair Flow property
5	**Thin Layer Chromatography Analysis Of Herbal Granules**	Rf values of spots of separated phytochemicals in granules matched with the Rf values of spots of phytochemicals of respective plant material	spots of separated phytochemicals of Jatamansi, Shankhapushpi, Ashwagandha, Wekhand, Brahmi are present in final granule formulation.
6	**Chemical Tests**	Steroids found absent	Carbohydrates, reducing sugars, Flavonoids, Alkaloids, Tannins, Glycosides present
7	**Stability Study at room temperature**	Sample is kept for three month stability study at room temperature	Granules found stable after three months based on morphology, microscopy and physicochemical as well as pharmaceutical parameters.

DISCUSSION

All individual five drugs of *Ashwagandhadi* Compound were identified after powder microscopy of *Ashwagandhadi* Compound. The ingredients present in the *Ashwagandhadi* compound were confirmed as *Brahmi* (*Bacopa monieri*), *Ashwagandha* (*Withaniasomnifera*), *Jatamansi* (*Nardostachysjatamasni*), *Shankhapushpi (Convolvulus pluricaulis), Vacha* (*Acorus calamus*). Further physiochemical analysis of *Ashwagandhadi* Compound was carried out. Organoleptic evaluation of Granules showed Light brown colored powder with characteristic odor and sweet taste. During Macroscopic Evaluation of Granules; Sugar crystals observed, No foreign particles, stones, insects are present in powder and no fungal contamination was observed. Microscopic Evaluation of Powder of Granules observed Lignified xylem, vascular bundle, Lignified fiber, cuticle with oil globules, starch grains and calcium oxalate crystals. Spots of separated phytochemicals of *Jatamansi*, *Shankhapushpi*, *Ashwagandha*, *Vacha*, *Brahmi* are present in final granule formulation on Thin Layer Chromatography Analysis**.** Carbohydrates, reducing sugars, Flavonoids, Alkaloids, Tannins, Glycosides were present and steroids were absent after undergoing the chemical tests. *Ashwagandhadi* Granules were found stable after three months based on morphology, microscopy and physicochemical as well as pharmaceutical parameters.

CONCLUSION

All the contents of *Ashwagandhadi* Compound subjected for identification and authentication were confirmed as *Brahmi* (*Bacopa monieri*), *Ashwagandha* (*Withaniasomnifera*), *Jatamansi* (*Nardostachysjatamasni*), *Shankhapushpi (Convolvulus*

pluricaulis), Vacha (*Acorus calamus*). As standardization is an essential part of to establish safety and efficacy of any formulation; *Ashwagandhadi* Compound was analyzed on the basis of physiochemical properties. It was found that formulation contains Carbohydrates, reducing sugars, Flavonoids, Alkaloids, Tannins, Glycosides which can be beneficial in various conditions including neuroprotective and cardioprotective effects. As *Ashwagandhadi* Compound is unique formulation prepared to increase its palatability in pediatric population; it was subjected to Physiochemical and HPTLC studies. It is inferred that formulation meets the minimum qualitative standards as reported in the API at preliminary level. Further animal studies and human clinical trials will establish its efficacy and safety for the benefit of society.

ACKNOWLEDGEMENT

Authors express their sincere gratitude to Prof. Milind Godbole, Hon'ble Principal, YAC, PhD Centre, Kodoli, and Dr. Sharada Deore, Asso. Prof. & Head Pharmacognosy Department, Government Pharmacy College Amravati, for their valuable technical inputs and encouragement for this work.

Conflict of Interest – None.

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