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ANALYTICAL EVALUATION OF AYURVEDIC FORMULATION USED IN MANAGEMENT OF PILES

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ABSTRACT

Piles are medically defined as the inflamed or expanded blood vessels in and around the anus which normally begins to bleed. Internal and External piles are generally seen types of piles. Treatment of piles is more about management of piles. Ayurveda offers various therapies for it. Variety of formulations in the form of creams, ointments, tablets and capsules are available in the market. Patients prefer taking tablets or capsules for instant relief from pain and irritation. Authors have selected an ayurvedic capsule formulation named Pylee capsule for the study. Since it contains ingredients which help in treating piles naturally, the formulation has got medicinal value, therefore its quality has to be monitored before administration. In this study attempts were made to analyze three lots of Pylee capsules for their physicochemical parameters. Active functional groups like Tannins, Curcumin, and Bitters were quantified as 48.218 to 61.710mg, 9.258 to 11.914mg, and 13.216 to 17.490mg per capsule respectively in three different lots. Presence of other ingredients was qualitatively proved by applying well versed HPTLC technique. Attempts were made to prove the quality of final product by comparing the concentration of major functional groups in final formulation against the same in corresponding active ingredients. Current study also gives the assurance about the safety with respect to the heavy metals and microbiological load in the sample which was found to be well within specified limits determined by AYUSH and USP /BP respectively.

INTRODUCTION

Nowadays due to the hectic lifestyle, stress level is going on increasing. We need to control the stress level for healthy survival. The purpose of life is four – fold[1], to achieve dharma (virtue), artha (wealth), kama (enjoyment) and moksha (salvation). In order to attain success in this four - fold purpose of life, it is essential to maintain life not only in a disease-free state but also in a positive healthy state of body,mind and spirit. The emphasis on the maintenance of positive health is a distinguishing feature of Ayurveda. Here Ayurveda contributes a lot. We should pay attention to basic activities such as regular breathing, eating, intake of sufficient quantity of food and water, sleeping well, exercising, and meditation. All these activities need to be given due importance. Dr. Nidhi Bathra in the editorial page of "Ayurveda and ALL" writes 'Eating the right kind of food is of prime significance. Ayurvedic recommendations for proper diet vary with each individual's constitution, the kind of environment you are living in, the season etc.'

Imbalanced diet, stress, obesity, leads to constipation which in turn some time gives rise to the most painful disease called "PILES". Piles are medically known as Hemorrhoids. It is a condition wherein the veins inside and outside the anus or lower rectum gets inflamed or swollen. Symptoms of piles include feeling of soreness, irritation or pain while passing stool. It is generally caused by faulty irregular diet and genetic tendencies. Constipation and piles are interrelated. Ayurveda offers therapies[2] like oleation and hydrotherapy for relief from piles. Various types of formulations like oils, ointments, creams, liniments, capsules, tablets are available in the market to cure piles. The ayurvedic medicine under study namely "Pylee capsule" is one of the effective formulations manufactured in our own laboratory. It contains ingredients which help in treating piles naturally. These ingredients are anti-inflammatory and anti-infective agents, which reduces inflammation and promotes healing. Laxative property corrects chronic constipation associated with hemorrhoids.

Each capsule contains *Azadirachta indica* extract, *Terminalia chebula* extract, *Symplocos racemosa* extract, *Curcuma longa* extract, *Glycyrrhiza glabra* extract. Chemical composition[3] recorded in the literature for each of this ingredient is – (1) *Azadirachta indica* – Bitters, rutin, (2) *Terminalia chebula* – shikimic, gallic, triacontanoic acids, beta-sitosterol, daucosterol, ellagitannin, punicalagin, teaflavin A, phloroglucinol, pyrogallol, (3) *Symplocos racemosa* – colloturine, loturidine, betulinic, oleanolic, ellegic acids, (4)*Curcuma longa* – curcumin, volatile oil, turmerones, (5) *Glycyrrhiza glabra* – glycyrrhizine, glycyrrhetinic acid, isoflavanoids, chalcones, lignans, amino acids, amines, coumarine.

Each ingredient has medicinal value. *Azadirachta indica* extract[4] possess antibacterial and antiseptic properties. Itsanti-inflammatoryproperty helps to control anal infection caused due to piles, thus relieves pain. *Terminalia chebula* fruit pulp exhibits laxative activity. Myrobalans[5] are a safe and effective purgative, astringent and alterative. It increases the frequency of stools and improves healthy bowel movement. It also helps to prevent hemorrhoids by promoting the elimination of waste and avoiding constipation. *Symplocos racemosa* is useful in treating bleeding piles and helps to arrest haemorrhage[6]. It also reduces pain and edema. *Curcuma longa* is a powerful anti-inflammatory[7], antiseptic agent. It is useful in allergic conditions, cough, asthama, constipation, anemia, and haemorrhages. It helps to stop bleeding. *Glycyrrhiza glabra* acts as a laxative[8] in combination with other drugs. It can be used as anti-inflammatory agent in peptic ulcer. Its anti-inflammatory property reduces the venous inflammation and correct anorectal piles disorder, generally advised to patients suffering from constipation.

Literature proves the medicinal value of all the active ingredients used in Pylee capsule for management of Piles. It is necessary to ensure the quality of batch (lot) to batch (lot) samples for its content and control the possible contaminants. Hence quality test protocol for this product is set and practical evaluation is carried out accordingly.

MATERIAL AND METHODS

All the reagents and chemicals used for manufacturing as well as testing purposes were of AR grade and were purchased from M/s Qualigens and M/s Merck India Ltd.

Active raw materials used in Pylee capsule formulation were procured from authorized vendors in India, quality and authenticity of which is ensured by carrying out relevant testing in our own laboratory.

All the glasswares used were well calibrated and were procured from M/s Borosil. Instruments used were weighing balance (M/s Schimadzu Corporation), hot plate, heating mantles (supplied by M/s Sunbim). Muffle furnace (M/s Pathak), UV-Visible double beam spectrophotometer (M/s Schimadzu) and HPTLC (M/s Anchrome, M/s Camag).

Method of analysis – General test parameters of capsule formulations such as average weight and disintegration time were carried out as per standard pharmacopoeial method[9].Other physicochemical test parameters such as loss on drying, total ash, acid insoluble ash, water and alcohol soluble extractives in active raw materials as well as in final capsule formulation were carried out as per the ayurvedic pharmacopoeia of India[10].Functional groups like tannins[11], bitters[12], curcumin[13]were carried out in respective raw materials and final

formulation by applying standardized documented methods. Crude glycyrrhizin was estimated in *Glycyrrhiza glabra* extract by gravimetric method (Garret method)[14]. Presence of all the active ingredients was qualitatively proved by using well versed HPTLC technique. For carrying out HPTLC, all active raw materials and blend of one capsule from each lot were extracted individually in methanol, 10 microlitre of each was spotted on TLC Aluminium sheet silica gel 60 F 254.The plate was allowed to run in (9:1) Chloroform : Methanol system previously saturated for 30 minutes. The plate was subjected to air drying after attaining the level of the solvent front upto the mark. Then it was scanned at 254 and 366nm. Also sprayed with vanillin sulphuric acid reagent and the presence of all label claimed ingredients was photodocumented using camag powershot camera unit. Heavy metal testing in final capsule formulation was carried out using Inductive Coupled Plasma. Microbiological tests were performed in all active raw materials as well as in capsule formulation as per the guidelines given in USP /BP.

RESULTS AND DISCUSSION

All the observations were documented in appropriate manner.

Table 1 : Composition of Pylee capsule

Label Claim: Each Capsule contains:

Sr. No	Indian Name	Botanical Name	Standardised for	Quantity in mg
1	Neem bark extract	*Azadirachta indica*	Bitters 2.0%	100.0
2	Haritaki extract	*Terminalia chebula*	Tannins 40.0%	100.0
3	Lodhra extract	*Symplocos racemosa*	Tannins 2.0%	50.0
4	Haridra extract	*Curcuma longa*	Curcumin 20.0%	50.0
5	Yastimadhu extract	*Glycyrrhiza glabra*	Glycyrrhizin20.0%	25.0
6	Excipients	----		q.s.

As per the label claim, approved raw materials with desired quality were used in formulating the Pylee capsule. Table 1 shows the quantity of active raw materials added as well percentage of different functional groups in corresponding raw materials.

Table 2 below focuses on the quality analysis of individual active raw material.

Table 2 : Analyticaltest summary of active raw materials

Sr. No.		***Azadirachta indica*** (Neem bark) ***extract***	***Terminalia chebula*** *(Haritaki)* ***extarct***	***Symplocos racemosa*** *(Lodhra)* ***extract***	***Curcuma longa*** (Haridra) **extract**	***Glycyrrhiza glabra*** (Yastimadhu) ***extract***
1	Description	Dark Brown powder , odor-characteristic , taste - bitter	Brown powder, taste - astringent	Brown colored powder	Orange yellow powder, odor – peculiar	Light brown powder, taste - sweet
2	Loss On drying at 105^0C (%w/w)	4.020	3.355	2.473	3.891	2.840
3	Total ash (%w/w)	16.960	4.764	7.334	35.951	5.226
4	Acid insoluble ash (%w/w)	0.550	0.392	0.495	NA	1.331
5	Water soluble extractive on d/b (%w/w)	81.910	93.044	96.413	NA	98.657
6	pH of 1%w/v solution in water	5.25	3.08	4.17	5.91	4.84
7	Assay (Functional group) (%w/w)	Bitter 4.010	Tannins 45.599	Tannins 4.168	Curcumin 23.706	Glycyrrhizin 22.007
8	**MICROBIOLOGICAL TESTING: → (As per USP/BP Guidelines)**					
i	Total Aerobic Microbial Count	570 cfu/g	60 cfu/g	40 cfu/g	<10 cfu/g	40 cfu/g
ii	Total Combined Yeast/Moulds Count	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
iii	Bile-Tolerant Gram Negative Bacteria	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
iv	*Escherichia coli*	Absent	Absent	Absent	Absent	Absent
v	*Salmonellae spp.*	Absent	Absent	Absent	Absent	Absent
vi	*Staphylococcus aureus*	Absent	Absent	Absent	Absent	Absent
vii	*Pseudomonas aeruginosa*	Absent	Absent	Absent	Absent	Absent
viii	*Clostridium spp.*	Absent	Absent	Absent	Absent	Absent

cfu/g : colony forming unit per gram

From table 2, it can be seen that *Azadirachta indica*(Neem bark) *extract* gives more than 100% bitters. Tannins in *Terminalia chebula* (Haritaki) extract and Symplocosracemosa (Lodhra) extract are found to be around 113.99% and more than 100.00% respectively. Curcumin in *Curcuma longa* (Haridra)is 118.53% of calculated value. Crude glycyrrhizin is found to be around 110.03%. All the raw materials are observed to be falling within the specified limit with respect to the microbiology.

Selvanet al[15] have also recommended phytochemical studies, pharmacognostical parameters and the pharmacological evaluation for the official standardization and anti-hyperlipedemic effects of the polyherbs. Similarly,physicochemical parameters of three different lots of Pylee capsule are documented. Specifications for final Pylee capsule formulation areset up with reference to the standardised value of corresponding concentrationof functional group in individual raw material.Theoretical values for Functional groups like Tannins,Curcumin and Bitters can be evaluated per capsule (With reference to table 1 and table 2) as-

1. Content of Tannins :Contribution for tannins from Haritaki extract standardized for 40% is, equivalent to 40.00mg.Lodhra extract for 2.00% is equivalent to1.00mg. Total 41mg tannins per capsule as per theoretical calculations. Hence specification for this parameter in final product is proposed as not less than 35.00mg/capsule. (which is lying between 80% and 90% of theoretical value.)
2. Content of Curcumin : The source of curcumn is*Curcuma longa*extract standardized for 20% curcumin. 50mg of which is used in each capsule, which is equivalent to 10mg. 80% of 10mg is 8.00. Hence the proposed specificationis Not Less Than 8.00mg/capsule.
3. Content of Bitters :Neem bark extract with more than or equal to 2.00% bitter is used. Since 100mg of this extract is used, it contributes minimum 2.00mg bitters per capsule.

Table 3A shows all the practical findings against the theoretical specifications.

Table 3A : Physicochemical parameters of Pylee capsule

SR. NO.	TEST PARAMETERS	SPECIFICATIONS	Lot 1	Lot 2	Lot 3
1	Description	"0" size hard gelatin capsule filled with yellowish to brown colored powder.	Complies	Complies	Complies
2	Disintegration Time (minutes)	Not more than 30.00	14.00	8.00	11.00
3	Average Weight of capsule (mg)	510.00 mg ± 7.5%	513.49	514.50	510.29
4	Average net filled weight of capsule (mg)	415.00 mg ± 7.5%	414.55	420.85	415.57
5	Uniformity of weight	± 7.5% of the average filled weight.	Complies	Complies	Complies
6	Loss On Drying at 105°C (%w/w)	Not more than 15.000	7.790	6.332	6.862
7	Total Ash (%w/w)	Not more than 20.000	7.690	10.798	11.271
8	Water Soluble Extractives (%w/w)	Not less than 30.000	59.025	53.035	52.910
9	pH of 1.00%w/v solution	4.00 to 7.00	4.16	4.20	4.00
10	Content of Tannins (mg/capsule)	Not less than 35.000	61.710	55.859	48.218
11	Content of Curcumin (mg/capsule)	Not less than 8.000	10.028	11.914	9.258
12	Content of Bitters (mg/capsule)	Not less than 2.000	13.216	17.490	14.840
13	HPTLC Fingerprinting profile	To pass the test-Complies/ Does not comply	Complies	Complies	Complies

Content of tannins vary fromabout 48.00 to 62.00mg per capsule because most of the herbal ingredients contain phenolic compounds and they also contribute here. Content of curcumin is observed to be between 9.00 to 12.00 mg/capsule which is equivalent to 90.00% to 120.00% of theoretical value. Similarly bitter content shows variation from 13.00mg to 18.00mg/capsule. Most of the other ingredients also add on bitter.

Table 3B : Heavy metals as per AYUSH guidelines

SR.NO.	TEST PARAMETERS	SPECIFICATIONS	Lot 1	Lot 2	Lot 3
i	Arsenic (as As)	Not more than 3 ppm	< 0.20ppm	< 0.20ppm	< 0.20ppm
ii	Mercury (as Hg)	Not more than 1 ppm	< 0.20ppm	< 0.20ppm	< 0.20ppm
iii	Lead (as Pb)	Not more than 10 ppm	< 0.20ppm	< 0.20ppm	< 0.20ppm
iv	Cadmium (as Cd)	Not more than 0.3 ppm	< 0.20ppm	< 0.20ppm	< 0.20ppm

< : Less than, ppm : parts per million

Table 3C : Microbiological testing as per BP/USP specifications

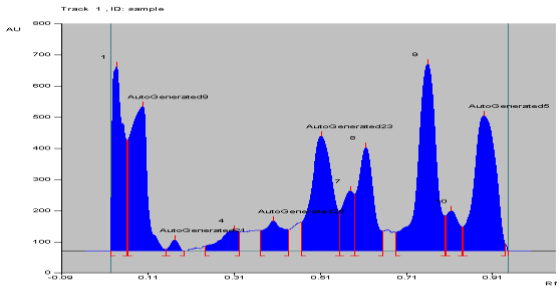
SR.NO	TEST PARAMETERS	SPECIFICATIONS	Lot 1	Lot 2	Lot 3
i)	Total aerobic bacterial count	Not more than 10000 cfu/g	90 cfu/g	40 cfu/g	40 cfu/g
ii)	Total combined yeast, and moulds count	Not more than 1000 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
iii)	Bile Tolerant Gram negative bacteria	Not more than 100 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
iv)	*Escherichia coli*	Should be absent in 10g	Absent	Absent	Absent
v)	*Salmonella spp.*	Should be absent in 10g	Absent	Absent	Absent
vi)	*Staphylococcus aureus*	Should be absent	Absent	Absent	Absent
vii)	*Pseudomonas aeruginosa*	Should be absent	Absent	Absent	Absent
viii)	*Clostridia spp.*	Should be absent	Absent	Absent	Absent

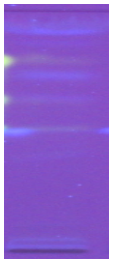
Cfu/g : Colony forming unit per gram

Table 3B gives clear picture about the heavy metals. Table 3C for microbiological load in all lots against the standardized value. None of the sample crosses the limit of any heavy metal, and Microbiological parameters, hence can be considered “safe.”

Qualitative determination of presence of all active ingredients are confirmed by carrying out HPTLC fingerprinting against the individual extract used in the formulations as shown in Figure 1 below.

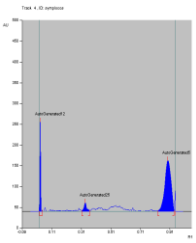
Fig.1 : HPTLC fingerprinting of Pylee capsule

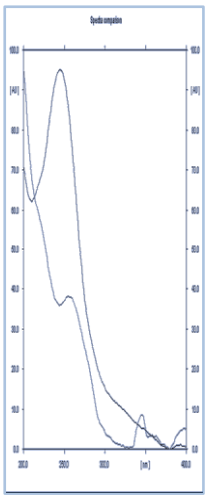
254nm	Pylee capsule	Rf values
		**0.04 0.51** **0.10 0.58** **0.17 0.62** **0.31 0.76** **0.40 0.81** **0.51 0.89**

Pylee capsule (Under 366nm)	***Azadirachta indica* extract**	**Rf Values**
		Blue spot at Rf about 0.85

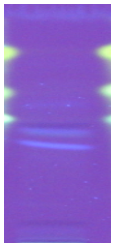
Pylee capsule (Under 254nm)	***Terminalia chebula* extract**	**Rf values**
		Dark spot at Rf about 0.09

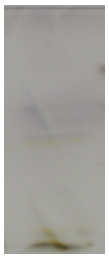
Pylee capsule (After spraying)	***Terminalia chebula* extract**	**Rf values**
		Blue spot at Rf about 0.26

254nm	***Symplocos racemosa* extract**	**Rf values**
		0.03 0.34 **0.90**



Pylee capsule (After spraying)	***Curcuma longa* extract**	**Rf values**
		Orange spots at Rf about 0.37, 0.46 & 0.64

Pylee capsule (Under 366nm)	***Glycyrrhiza glabra* extract**	**Rf values**
		Blue spots at Rf about 0.33 & 0.35

Pylee capsule (After spraying)	***Glycyrrhiza glabra* extract**	**Rf values**
		Yellow spots at Rf about 0.05 & 0.35

PREPARATION OF SAMPLE AND EXTRACTS USED AS REFERENCE STANDARD FOR SPOTTING

1= Pylee capsule : Blend of 1 capsule to 10 ml Methanol

2= *Azadirachta indica*extract : 100.0mg to 10ml Methanol

3= *Terminalia chebula* extract : 100.0mg to 10ml Methanol

4= *Symplocos racemosa* extract : 50.0mg to 10ml Methanol

5= *Curcuma longa* extract : 50.0mg to 10ml Methanol

6= *Glycyrrhiza glabra*extract : 25.0mg to 10ml Methanol

INFERENCES:

- *Azadirachta indica* extract : After observing the plate under 366 nm, blue colored spot is observed at Rf about 0.85 in both extract and sample. This indicates the presence of *Azadirachta indica* extract in the sample.

- *Terminalia chebula* extract : After observing the plate under 254 nm, dark colored spot is observed at Rf about 0.09 in both extract and sample. After spraying the plate with VSR and observing under white light, blue colored spot is seen at Rf about 0.26, in both extract and sample. This indicates the presence of *Terminalia chebula* extract in the sample.
- *Symplocos racemosa* extract: After scanning the plate at 254 nm, overlapping spectrum observed at Rf about 0.90 in extract and Rf about 0.89 in the sample. This indicates the presence of *Symplocos racemosa* extract in the sample.
- *Curcuma longa* extract : After spraying plate with VSR and observing under white light, orange colored spots are seen at Rf about 0.37, 0.46 and 0.64 in both extract and sample.This indicates the presence of *Curcuma longa* extract in the sample.
- *Glycyrrhiza glabra* extract : After observing the plate under 366 nm, blue colored spots are observed at Rf about 0.33 and0.35 in both extract and sample. After spraying the plate with VSR and observing under white light, yellow colored spots are seen at Rf about 0.05 and 0.35, in both extract and sample. This indicates the presence of *Glycyrrhiza glabra* extract in the sample.

There are several good reasons why herbal medicines tend to be so safe[16]. The complex mixture of chemical constituents of plants includes substances like the tannins and saponins which tend to moderate the activity of the more active chemicals. This is the effect of the total action of the mixure of plant ingredients being very different from the action of isolated or concentrated chemicals. Likewise all active ingredients used in Pylee capsule formulation are purely herbal that is natural origin. They are not only contributing with respect to isolated functional group but also gives synergetic effect to achieve overall medicinal effect.

CONCLUSION

All active ingredients used in the composition of Pylee capsule are authentic and medicinally useful as per ayurvedic literature. Chemically they are proved to be quality oriented. HPTLC analysis gives clear picture of presence of all ingredients. Specifications for final formulation is set up on the basis of corresponding functional groups like Bitters, Curcumin and Tanninsas per their concentrations in respective active components. Findings from batch to batch are evaluated and matched with the final specifications for compliance. Hence “Pylee capsule” seems to be quality product and can be recommended for management of Piles.

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