



### REVIEW ARTICLE

#### A REVIEW ON ANALYTICAL STUDIES AND PHARMACOLOGICAL EVALUATION OF POLYHERBAL FORMULATIONS

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#### Abstract

In standardization process, quality and purity of crude drugs are evaluated by various parameters. For herbal formulations, along with the chemical, physical, phytochemical, in- vivo and in- vitro parameters, it is significant to determine its quality standards. In traditional system of medicine, the developments of analytical parameters are an important step in establishing quality of products, presence of phytoconstituents and its therapeutic efficacy. In this review, Avipathy and Avipattikar churna, both are polyherbal formulations having nine similar herbal ingredients (Sunthi, Marica, Pippali, Amlaki, Musta, Vidanga, Elaichi, Patra, Triviti) but they are different in terms of indication and method of administration. Avipathy churna is targeted mainly to achieve pitta balance whereas Avipattikar churna for all the three dosha specified in Ayurvedic system of medicine. This review provides data on quality control and analytical studies such as Gas Chromatography coupled with Mass Spectrometry (GC-MS), High Performance Thin Layer Chromatography (HPTLC), Atomic Absorption Spectroscopy (AAS), Ultraviolet spectroscopy (UV) and pharmacological evaluation of Avipathy and Avipattikar churna. The analytical study shows the presence of various marker compounds. This helps to draw new conclusions from existing data to identify research areas. They assure the quality, purity and safety of herbal formulations.

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#### Introduction:-

According to World Health Organization, most of the present medications are derived from the herbs which are used traditionally as medicine. Plants with medical importance impart an important role in the development of new drugs. Their standardization and safety evaluation are the most important part. The lack of quality assurance is the problem facing by traditional systems of medicine even though they are effective.<sup>[1]</sup> This review is focused on polyherbal formulations Avipathy churnam and Avipattikar churnam which have Piper nigrum, Zingiber officinale, Emblica officinalis, Piper longum, Cyperus rotundus, Operculina turpethum, Embellia ribes, Cinnamomum tamala, Elettaria

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cardamomum as ingredients in both churna. A famous Ayurvedic herbal powder Avipathy churna is used in the treatment of pitta imbalance disorders such as sun burn, gastritis, migraine, excessive burning, sensation, dizziness etc. It is also used for liver disorders such as jaundice. The dose is 1-3 grams once or twice a day, before or after food, or as directed by physician. It consists of one part each of powders of Zingiber officinale, Piper longum, Piper nigrum, Cinnamomum zeylanicum, Elettaria cardamomum, Cinnamomum tamala, Cyperus rotundus, Emblica officinalis, Embellia ribes and Operculina turpethum. It is manufactured by Kottakkal arya vaidya sala, Nagarjuna Ayurvedic group, Ashoka pharmaceuticals, Vaidyaratnam oushadhasala and Sitaram Ayurveda pharmacy. [2] Avipathy churnam has been analysed by Gas chromatography Mass spectroscopy (GC-MS). The gastroprotective activity estimates that it can be used to treat ulcer. The laxative effect of Avipathy churnam and its modification as tablet were compared. The clinical study was based on combined effectiveness of Avipathy churnam and Chitrakadi vati in the treatment of hypothyroidism.

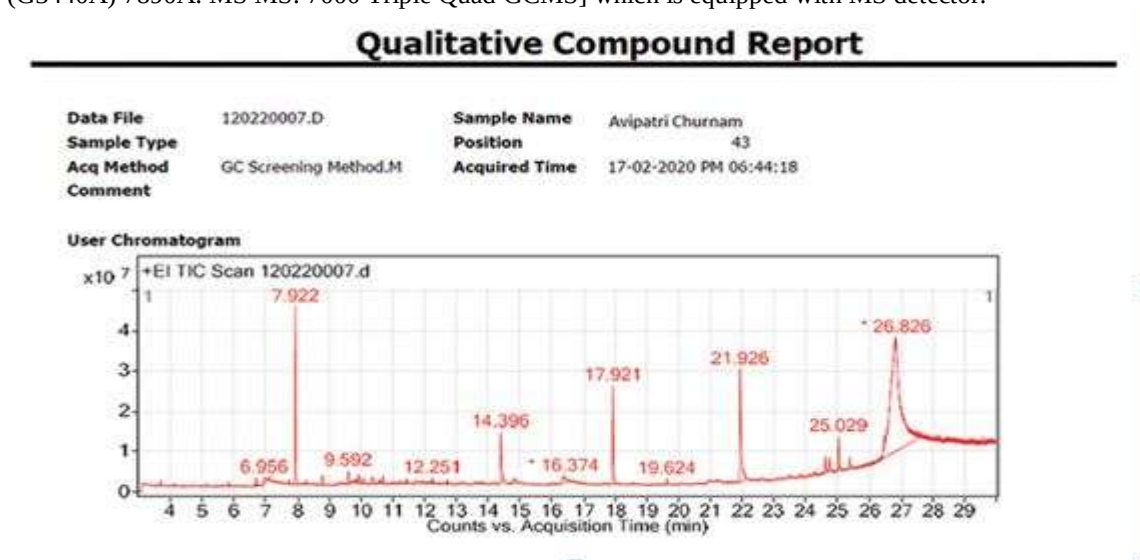
Avipattikar churna is an Ayurvedic herbal medicine which is used for hyperacidity, gastritis, loss of appetite and indigestion linked with piles, gastritis, urinary problems, difficulty in micturition and kidney stones. The important therapeutic uses are constipation, dyspepsia, haemorrhoids, and retention of urine, digestive impairment and urinary disorders. In supporting the functions of digestive tract and relieving symptoms of these diseases, the medicinal herbs in churna helps. The companies that manufacture this formulation such as Dabur, Baidyanath, Swadeshi, Baba Ramdev, Dhanvantri and so on. It contains 14 different herbs, sugar and salt in a specific ratio. The chief ingredient is Indian Jalap. It is administered twice a day after meals. [3] The standardization and quality control measures have been done for Avipattikar churna. Stability study was performed to confirm whether phytoconstituents in raw materials and formulation are same and to confirm the presence of eugenol after regular interval of time. The estimation of piperine by HPTLC and heavy metals and trace elements by Atomic Absorption Spectroscopy has been carried out. Quantitative analyses by Energy Dispersive X-ray Spectroscopy and UV spectroscopy in quality control were performed. It is having different pharmacological activities such as anti-secretory and anti-ulcerogenic against gastric ulcer. Gastroprotective action and antioxidant activity were also proved. The comparative clinical study with Patoladi kwath confirms that churna was more significant and better in effect. The additive effect also shows significant improvement in administration of drugs.

### Avipathy Churnam

#### GC-MS

The traditional medicines are made by a number of plants, plant parts which are in different proportion. The mechanism of action on any particular disease can be understood from the knowledge of biomolecules present in any Ayurvedic medicine. The medicinal role of Avipathy churnam can be identified from the GC-MS analysis. This is vital to standardize the formulation and make them more efficacious. [4]

In this study, Avipathy churnam was obtained from standard Ayurvedic vendor at Chennai. They used GC [Agilent GC: (G3440A) 7890A. MS MS: 7000 Triple Quad GCMS] which is equipped with MS detector.



**Figure 1:- GC-MS profile of Avipathy churnam.**

The retention values, types of possible compounds, their molecular formulae, molecular weight, peak area and their medicinal roles of each compound are identified (**Fig.1**). The result shows the identification of metabolites, which was accomplished by comparison of fragmentation pattern and retention time with mass spectra in NIST (National Institute of Standards and Technology) Spectral library stored in computer software of GC-MS. From this it is clear that Avipathy churnam have medicinal value ranging from anti-oxidant to hormonal balance. It concludes the authenticity of Avipathy churnam as a good digestive medicine. <sup>[5]</sup>

### Gastroprotective activity

Peptic ulcer is a major health hazard in terms of mortality and morbidity. When the stomach acid damages the lining of digestive tract, peptic ulcer occurs. The epithelium will expose to gastric acid if gastric mucosal protection is impaired which cause cell damage that leads to ulceration. <sup>[6]</sup>

Several reports have been shown that drugs such as H<sub>2</sub> blockers, proton pump inhibitors lead to adverse effect, drug interaction and increased incidence of relapses during ulcer therapy. Hence, to find an ideal anti-ulcer drug the search is extended to herbal drugs for better protection, easy availability, low cost and low toxicity. <sup>[7]</sup>

Types of ulcers are gastric ulcer, duodenal ulcer, and esophageal ulcer. When the peptic ulcer is in stomach, it is called gastric ulcer. When it is in duodenum it is duodenal ulcer. <sup>[8]</sup> The main causes of ulcers are infection, neuroendocrine factors, local mucosal factors, acids and pepsin. Infection caused by H-pylori is responsible for 80% to 90% of all peptic ulcers. Factors such as stress, nervous tension and endocrine disorders are implicated in pathogenesis. NSAID (Non-Steroidal Anti-inflammatory Drugs) reduce secretion of prostaglandin and damage mucosal barrier. When the balance between acids and defensive mucus layers are broken, ulcer <sup>[9, 10]</sup> occurs.

The gastroprotective activity was performed by two methods such as screening of antiulcer activity by ethanol induced gastric ulcer and by pylorus ligation method. Avipathy churnam possess anti-ulcer activity in two experimental models. The mechanism of action is the direct neutralization of acid. It is also related to the reduction in gastric volume. But k<sup>+</sup> contents reflect exchange mechanism for H<sup>+</sup> in parietal cell by H<sup>+</sup> K<sup>+</sup> ATP ase. The result shows that it is a good and safe therapeutic agent for ulcer treatment. Ranitidine and Pantoprazole were used to predict the nature of action.

Final results confirm that Avipathy churnam and ranitidine were equal and comparable. So it has H<sub>2</sub> receptor antagonistic effect. Hence, it concludes that Avipathy churnam can be used to treat ulcer without any harmful effects. <sup>[11]</sup>

### Modification of Avipathi churnam

Dr. Prathap Varma reported a study in his thesis. This study is based on pharmaceutical modification of Avipathy churnam and their pharmacological evaluation. The work includes pharmaceutical study, analytical study and pharmacological study. In pharmaceutical study, three samples ie, churnam, vati and syrup were prepared according to classical method. In analytical study, these were chemically analyzed and verified. In pharmacological study, evaluation of laxative effect on Avipathy churnam, Avipathy tablet and Avipathy syrup on Swiss albino mice were done.

Initially Avipathy churnam was prepared and then the modified version ie Avipathy tablet and Avipathy syrup was prepared by using necessary adjuvants. Then organoleptic characteristics and chemical analysis were observed for each. TLC for three samples was taken and observed same R<sub>f</sub> value for churnam, tablet and syrup. Then they were experimentally evaluated for the laxative effect of different forms of Avipathy churna by conducting charcoal meal test (Intestinal motility test) on Swiss albino mice.

Preparation of churnam was done by placing the drugs mentioned in formulation into micro-pulverizer for obtaining fine powder. Then the granules were prepared and it was kept in drier for removing water content. Granule form of drug was compressed with the help of rotary punching machine and tablets were prepared. Starch and gum acacia were used as active ingredient. In preparation of syrup, a two-step procedure was followed.

1. Preparation of Kashaya
2. Preparation of Avipathy syrup

After proper cleaning and drying of raw drugs, coarse powder was prepared and 26.9 L of water was added and concentrated to 3.36 L. The sugar candy was added and heated till 60% brix value was obtained. The analytical study results show that churnam possess 3.41% w/w ash and 0.2% w/w of acid insoluble ash. The hardness of tablet was 3 kg/cm<sup>2</sup> and moisture percentage was 3.6% w/w. The pH of syrup obtained as 4.770 and specific gravity 1.290. In pharmacological study, the laxative effect of test formulations was compared. The index of activity was based on the distance travelled by charcoal meal. Higher the distance travelled; higher would be the laxative activity. From the results, it was observed that churnam have marginal increase in intestinal motility which indicates mild laxative action. Tablet produced a non- significant decrease in intestinal motility indicating intestinal relaxation. A high decrease in intestinal motility was observed for therapeutic dose of syrup. At higher doses, it would have overcome the intestinal motility by reducing activity of other ingredients. Thus, this study shows that syrup wouldn't be proper formulation to produce laxative effect. Hence it was found that churnam produce better laxative effect than tablet and syrup. <sup>[12]</sup>

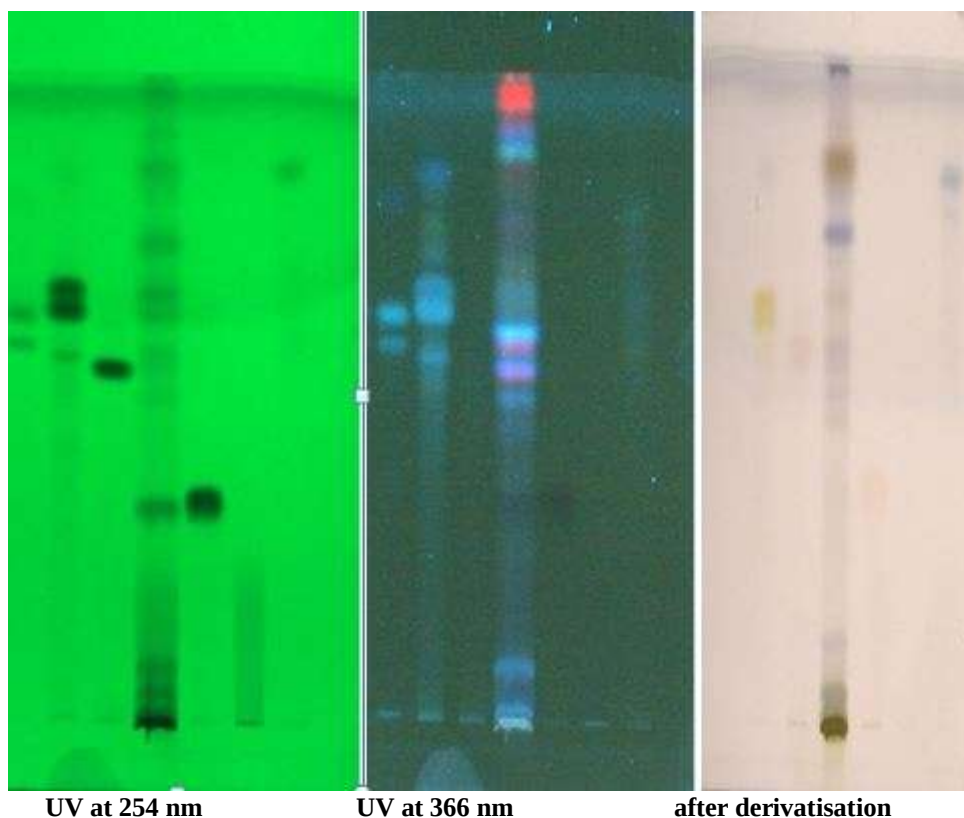
### Clinical study

This was carried out to study the combined effectiveness of Avipathy churnam and Chitrakadi vati in the management of Hypothyroidism. This study was about an open labeled single arm clinical study at outpatient department level with pre and post design, convenient sampling and sample size of 23 hypothyroidism patients. After the preparation of chitrakadi vati and avipathy churnam, treatment was done. Chitrakadi vati was administered with warm water half an hour before food as one tablet twice daily for 84 days in combination with avipathy churnam 12 gm with honey at early morning in empty stomach once in a month. It should be continued for three consecutive months. By the help of a suitable clinical scoring of hypothyroidism-“BILLEWICZ SCORE”, the assessment of subjective parameters were done on 1<sup>st</sup> day, 10<sup>th</sup> day, 41<sup>st</sup> day, 71<sup>st</sup> day and 90<sup>th</sup> day. The symptoms observed were cold, dry skin, peri orbital puffiness, diminished sweating, constipation, weight increase, and deafness. The signs were coarse skin, cold skin, slow movements, and ankle jerk. On the basis of changes in the laboratory parameters like body weight and thyroid profile with respect to BMI, assessment of objective parameters were done on 1<sup>st</sup> and 90<sup>th</sup> day. In this study, from the fifty nine patients screened, twenty three patients were registered and twenty have completed their course of treatment. With the administration of Chitrakadi vati and Avipathy churnam, improvements were detected on subjective and objective parameters. Their combination also helped in relieving weight gain in ten patients and periorbital puffiness in twelve patients and cold intolerance in 11 patients. They show reduced serum TSH level from a mean value of 45.91 µ IU/ml before treatment to 11.98 µ IU/ml after treatment. They also show reduced BMI and Cross Billewicz Score between 10<sup>th</sup> and 41<sup>st</sup> day where maximum result was obtained. There were no difference observed in symptoms and signs such as hoarseness of voice, bradycardia and deafness, slowness of movement and sluggishness of angle jerk. Both drugs increased the level of serum T<sub>4</sub> and decreased the TSH level after 90 days of treatment. But, after 90 days of treatment there were no difference in BMI and serum T<sub>3</sub> levels. Hence the results confirm that administration of 12 gm Avipathy churnam with honey at early morning in empty stomach and 1 tablet (250 mg) Chitrakadi vati twice daily (morning and evening) with warm water before food once in a month is effective for the control of hypothyroidism. <sup>[13]</sup>

### Avipattikar Churnam

#### HPTLC fingerprint profile

In this study, main parameters of drug standardization and HPTLC fingerprint profile of Avipattikara churna is carried out. This was based on organoleptic evaluation, physical characteristics, physico chemical studies such as pH, acid insoluble ash, total ash, loss on drying at 105°C, extractive values etc and microbial load as per API, IP, WHO and AOAC guidelines. Avipattikar churnam was prepared as per (AFI) Ayurvedic Formulary of India part I. Then analysed their organoleptic character which was observed as light brown fine powder having characteristic odour and sweet pungent taste. The physical characteristics such as bulk density (0.3448 gm/cm<sup>3</sup>), tap density (0.5263 gm/cm<sup>3</sup>), hausner ratio (1.5263) and Carr's index (34.48 %) were obtained. The pH of Avipattikar churna was 4.95. <sup>[14]</sup> The physico chemical parameters such as loss on drying (3.92% w/w), ash value (4.39% w/w), acid insoluble ash (0.15% w/w), water soluble extractive (61.59% w/w) and alcohol soluble extractive value (20.95% w/w) were determined. <sup>[15,16,17]</sup> HPTLC fingerprint has been done using Toluene: Ethyl acetate: Formic acid (5:5:0.5 v/v/v) as solvent system at UV 366 nm, UV 254 nm and after derivatisation with vanillin sulphuric acid (**Fig.2**). The marker compounds used in this study were piperlongumine, piperine, syringic acid, gallic acid and 6- shagoal. The ethanol extract of Avipattikara churna shows presence of these marker compounds. In microbial contamination, bacterial load and fungal load were estimated. <sup>[18, 19, 20]</sup> The study revealed that total fungal count and total bacterial count were found within the limit.



**Figure 2:-** HPTLC profile of Ethanol extract of Avipattikara churna and Marker compounds at UV 254 nm, UV 366 nm and after derivatization.

#### Heavy metal and trace element analysis

This was based on the quantitative analysis of toxic heavy metals and essential trace elements by Atomic Absorption Spectroscopy. The study includes screening of 19 popular herbal churna preparations. Heavy metals like calcium, magnesium, aluminum, copper, zinc were determined using Flame Atomic Absorption Spectrometer. Arsenic and Mercury are heavy metals which are determined by hydride generation technique. Out of these 19 samples, Avipattikara churna has the lowest concentration. The concentration of magnesium was obtained with a range of 1047-1057 ppm and that of aluminum in the range of 384.3-387 ppm. Out of 19 samples, 16 samples for mercury content and 8 samples for lead content were exceed the WHO permissible limits. Cadmium was above the limit.

The result confirms that there is immediate need for quality control of churna formulations to ensure safety and efficacy. The adverse effects and the pharmacological – toxicological profile by impurities in dosage forms determine the safety. Hence it needs to maintain toxic metal content at minimal level. <sup>[21]</sup>

#### Quality control and stability study

An Ayurvedic medication Avipattikara churna is used for treating gastrointestinal complaints. For the management of constipation and gastritis, it is beneficial. Also it is used to treat urinary disorders, hyperacidity, gastritis, burning ache, piles, difficulty in micturition and kidney stones. Preparation of Avipattikar churna by using raw materials comply with the pharmacopoeial quality was done as per the composition (**Table 1**).

**Table 1:-** Composition of Avipattikar churna.

| Sl. No | Ayurvedic Name | Botanical Name       | Quantity |
|--------|----------------|----------------------|----------|
| 1      | Sunthi         | Zingiber officinale  | 1 part   |
| 2      | Marica         | Piper nigrum         | 1 part   |
| 3      | Pippali        | Piper longum         | 1 part   |
| 4      | Haritaki       | Terminalia chebula   | 1 part   |
| 5      | Bibhitaki      | Terminalia bellerica | 1 part   |



|    |         |                       |        |
|----|---------|-----------------------|--------|
| 6  | Amlaki  | Phyllanthus embellica | 1 part |
| 7  | Musta   | Cyperus rotundus      | 1 part |
| 8  | Vidanga | Embellia ribes        | 1 part |
| 9  | Elaichi | Elettaria cardamom    | 1 part |
| 10 | Patra   | Cinnamomum tamala     | 1 part |
| 11 | Lavanga | Syzygium aromaticum   | 1 part |
| 12 | Trivit  | Operculina turpethum  | 1 part |
| 13 | Vida    | Vida lavana           | 1 part |
| 14 | Sarkara | Saccharum officinarum | 1 part |

The organoleptic evaluation shows that it has burdy wood colour, and no specific taste and odour. It helps to identify the formulation. Physicochemical study provides standards to determine the quality. The parameters such as bulk density (0.542 gm/ml), tapped density (0.724 gm/ml), hausner ratio (1.952), compressibility index (15.8%) and total ash (0.039% w/w) were obtained. The presence of various therapeutically active constituents was understood from phytochemical evaluation. It reveals the presence of tannins, steroids, saponins and phenolic compounds. Their presence and absence depends on raw materials and procedure used. The biochemical test such as carbohydrates, proteins, fats, and starch were performed from which all tests were positive except fats. Hence, the presence of different chemical compounds is useful for treating ailments. The presence of marker compound Gallic acid was checked by hyphenated techniques like HPTLC and HPLC for assessing its quality. The HPTLC fingerprint of Avipattikar churna is represented in **(Fig.3)**. Even though, the presence of plant constituents in raw materials as well as formulation can be visualized for monitoring their quality, marker compound can act as a fingerprint.



**Figure 3:-** HPTLC fingerprint of Avipattikar churna.

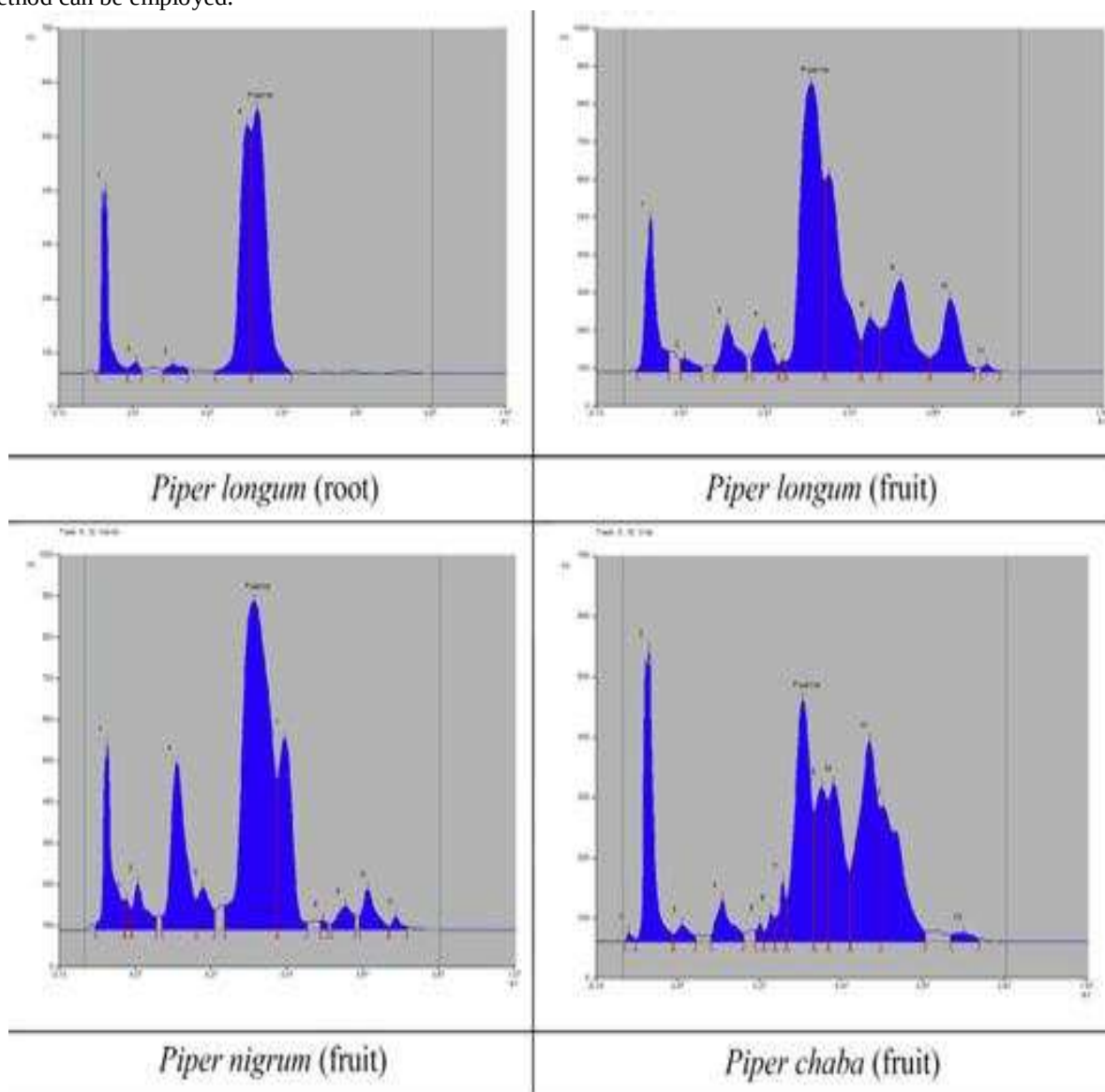
To confirm whether phytoconstituents present in raw materials and formulation are same, stability studies were carried out upto 45 days and also to confirm presence of eugenol after regular interval of time. <sup>[22]</sup>

#### Piperine estimation by HPTLC

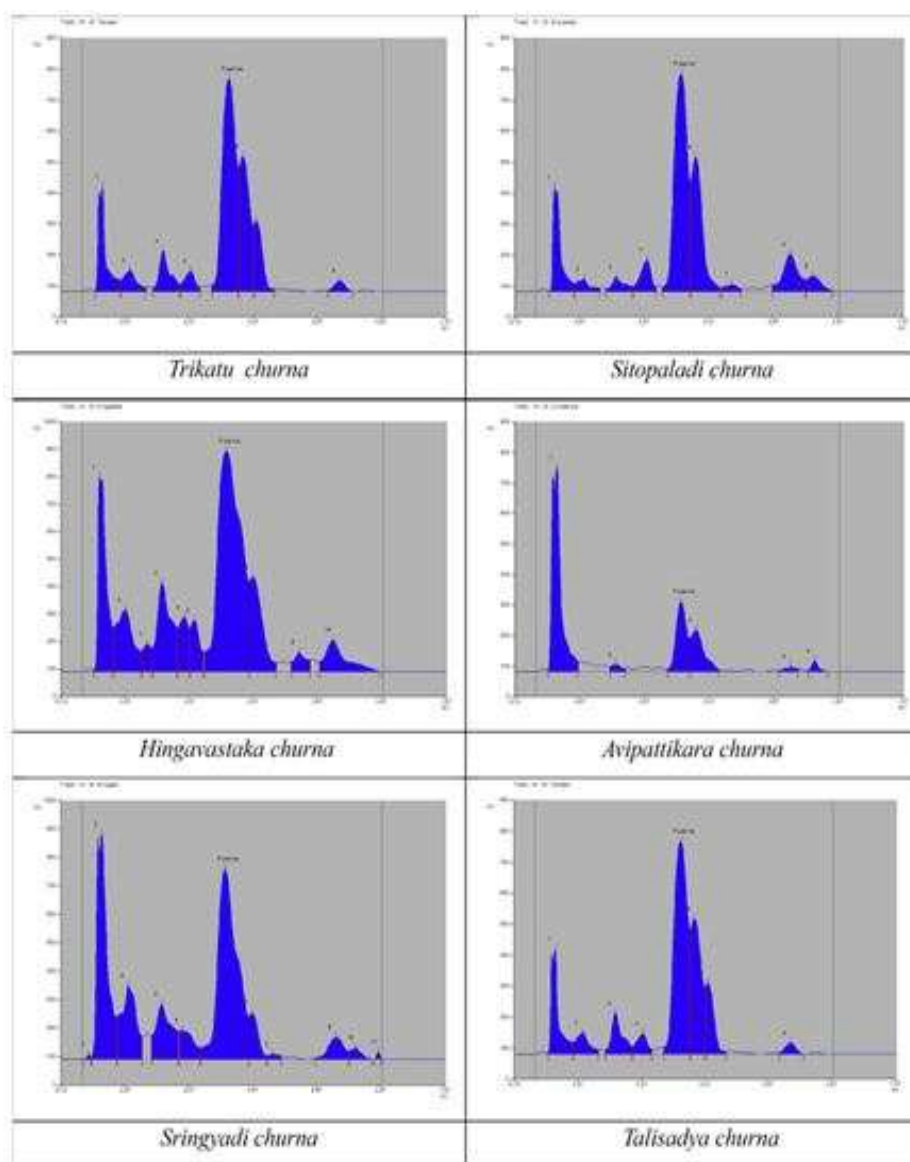
This was based on the quantification and detection of marker compound piperine in Ayurvedic formulations like Avipattikar churna, Trikatu churna, Talisadya churna, Sitopaladi churna, Sringyadi churna and Hingavastaka churna by using an accurate, simple, and sensitive HPTLC method. These were commonly used to treat fever, cold, indigestion, asthma, and cough, inflammatory and respiratory disorders. <sup>[23]</sup> In this group of herbs, the active principle is piperine (an alkaloid). The piperine content is contributed by the ingredients from piperaceae family.

Fruit from piper nigrum, stem from piper chaba and root and fruit from piper longum as raw material and the formulations were used as test samples. The methanolic extract of churna and raw materials was taken for HPTLC. The solvent system toluene: ethyl acetate in the ratio 7:3 was used. The standard piperine has  $R_f$  value 0.39. By using the calibration curve of standard piperine, the piperine content in samples were calculated and expressed as mg/g of dry samples. HPTLC chromatograms of piper species **(Fig.4)** as well as churna formulations **(Fig.5)** were illustrated. Piper longum fruits have  $1.48 \pm 0.95$  mg/g. Piper nigrum fruit contain  $1.94 \pm 0.77$  mg/g and  $0.22 \pm 0.61$  mg/g in piper chaba stem. These were found to be within standard limit.

The physicochemical parameters for Ayurvedic formulations and each raw material were evaluated for quality standards as mentioned in Ayurvedic Pharmacopoeia of India. [24] Maximum piperine was quantified in Hingavastaka churna ( $7.09 \pm 0.73$  mg/g) followed by Sringyadi churna ( $3.62 \pm 0.58$  mg/g), Sitopaladi churna ( $2.81 \pm 0.52$  mg/g), Talisadya churna ( $2.62 \pm 0.64$  mg/g), Trikathu churna ( $2.27 \pm 0.83$  mg/g and Avipattikar churna ( $0.16 \pm 0.43$  mg/g). The quantities and composition of piper species vary from each other in Ayurvedic churnas. This becomes the primary to quantify piperine in Ayurvedic formulations as well as single herbs. Hence it can be concluded that for quantitative analysis and standardization of piperine in raw herbs as well formulations, HPTLC method can be employed. [25]



**Figure 4:-** HPTLC Chromatogram of single herbs (piper species).



**Figure 5:-** HPTLC chromatogram of Ayurvedic churna formulation.

### X – Ray Spectroscopy

CCRAS has given direction for standardization of formulations. But it is essential to develop methods by using phytochemical markers for batch uniformity in production of Ayurvedic formulations. A technique called Energy Dispersive X-ray Spectroscopy (EDX) is used for elemental analysis and characterization of a sample. Different sample types such as powder, solid, liquid, metals etc. can be analyzed with simple sample preparation over a wide range of concentration. To quantify and identify the trace elements in a sample EDX is used as a new technique. The trace elements and salt in formulations helps to develop the therapeutic efficacy of main drugs. Hence, EDX can be used as a major tool for standardization.

Avipattikar churna is used in digestive and irritable bowel disorders. Vida lavana (black salt) - one of the ingredient which contains small amount of trace elements like magnesium and iron. It helps to replenish the salt lost during exercise. This was based on the quantification of various trace elements of in house formulation, two different marketed formulations and a formulation without salt of Avipattikar churna by EDX and flame photometry. The quantity of present trace elements in marketed formulations, in house formulations and in house without salt is depicted in (Fig. 6, 7, 8 and 9) respectively. This study was also based on the comparison of potassium and sodium



by EDX and flame photometry. In EDX besides sodium and potassium other trace elements such as calcium, copper, zinc and chloride were detected and quantified.

From the flame photometry results, the ranges of sodium were obtained from 0.1142% to 0.3812% weight and potassium shows from 0.322% to 0.473%. From EDX, sodium shows range from 0.50% to 0.58% weight and potassium from 0.34% to 0.82% weight. The result also reveals the presence of chlorine, potassium, sodium and other trace elements in the in-house formulation without salt which indicates the existence of trace elements other than salt. EDX gives better understanding of trace elements. The results from both EDX and flame photometry show quite a difference, but the findings from EDX showed higher accuracy, sensitivity and precision. <sup>[26]</sup>

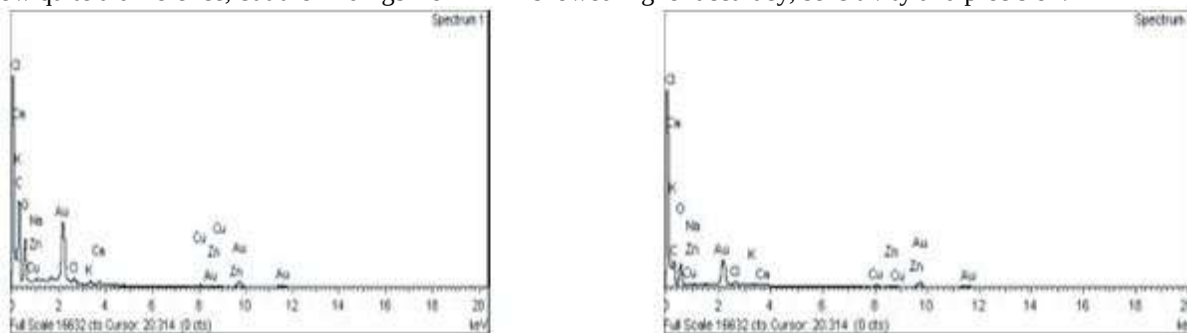


Figure: - 6 and 7.

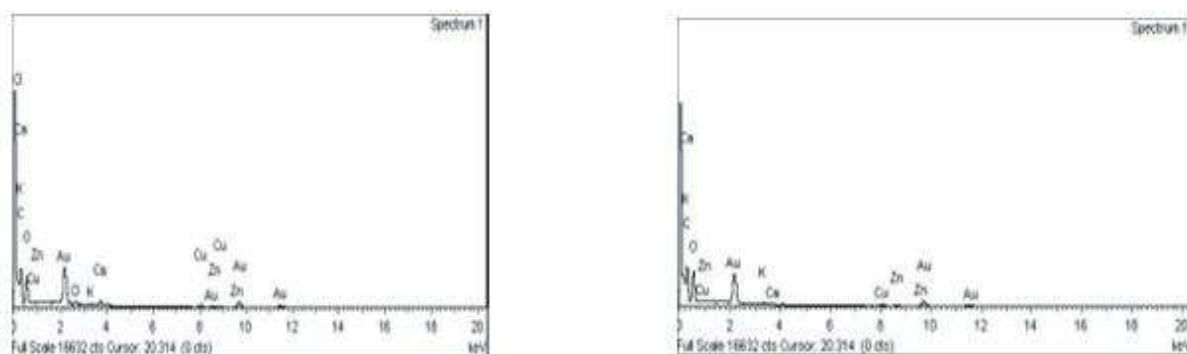


Figure: - 8 and 9.

Figure 6,7,8,9:- Graph showing quantity of trace elements in formulation A, B, in-house and in house without salt respectively.

#### Quantitative analysis by UV- Spectroscopy

In this research work, Avipattikar churnam has been prepared as per Bhaishajya Ratnawali, Sastriya Siddha prayanak and Aurvedic Formulary of India. <sup>[27]</sup> Here the marker compound gallic acid was used for developing UV standardization of Avipattikar churnam. <sup>[28]</sup> This work was performed for estimation of gallic acid by using methanolic extract of Avipattikar churnam.

For the Gallic acid (marker compound) determination which is the major constituent of Avipattikar churna and its ingredients, UV Spectroscopy analysis method was developed. <sup>[29,30]</sup> Three batches of churnam AVI-1, AVI-2, AVI-3, MAVI (marketed formulation) and its ingredients (Harde, Amla and Bahera) bearing gallic acid were estimated for the presence marker compound.

The standard stock solution of Gallic acid and its calibration curve was prepared. Precision and accuracy analysis was done. The precision can be expressed as relative standard or standard deviation of a series of measurements. Accuracy is laid out by spiking working standard in placebo and recovery study at levels 80, 100 and 120% of the working standard. For intraday precision UV spectroscopy experiments were repeated 5 times (1<sup>st</sup> hr, 2<sup>nd</sup> hr, 3<sup>rd</sup> hr and 4<sup>th</sup> hr) and 5 day for interday precision.

Estimation of gallic acid was performed at 271 nm and by spiking known amount of Gallic acid its recovery study was done on Avipattikar churnam at different levels of working standard.

The results confirm that the determination of marker compound gallic acid were carried out in AVI-1, AVI-2, AVI-3, MAVI and its ingredients (Harde, Amla, Bahera) bearing gallic acid. The parameters used were Absorbance maxima wavelength (271nm), Beer's Law limit (2-7 µg/ml), accuracy (%) as 98.87 and regression analysis equation  $y = 0.164x - 0.23$ . The gallic acid content in Amla, Harde, Bahera, AVI-1, AVI-2, AVI-3 and MAVI were obtained as  $3.213 \pm 0.011$ ,  $3.481 \pm 0.015$ ,  $7.334 \pm 0.021$ ,  $0.312 \pm 0.001$ ,  $0.242 \pm 0.001$ ,  $0.233 \pm 0.002$ ,  $0.232 \pm 0.001\%$  w/w respectively. The percentage recovery study of interday was 0.587 and 0.129 for intraday. The mean value of recovery study was 98.875. Hence it can be concluded that the standards established are precise, accurate, efficient and sensitive which can be used as reference by Ayurvedic manufactures. <sup>[31]</sup>

### Standardization and its parameters

This study reported the standardization of in house preparation and two marketed formulation based on physical characteristics, organoleptic characters and physico-chemical properties. As per the procedure given in the Ayurvedic Formulary of India the churna was prepared. The marketed samples were of Baidyanath (B) and Dabur (D). The alcohol soluble, water soluble extractive values and ash values of individual ingredients of Avipattikar churna were estimated as described by WHO guidelines. The organoleptic and physicochemical comparisons between in house and marketed formulations were found to be similar and variation was insignificant. The pH of three formulations was comparable and was slightly acidic. The sodium content in different formulations was also comparable and Baidyanath formulation was found to be highest in sodium content compared to in house and Dabur. The fluorescence analyses of three formulations are also reported. <sup>[32]</sup>

**Vihangesh Kumar Dixit et al.** were carried out the standardization parameters for herbal formulation Avipattikar churnam. <sup>[33]</sup> It was in terms of loss on drying analysis, organoleptic evaluation of ingredients with rasayana formulation, phytochemical screening, foreign matter and evaluation of extractive value, ash value and rheological evaluation. These were evaluated for Avipattikar churna (AVI-1, AVI-2, and AVI-3) and MAVI (marketed formulation). The organoleptic evaluation of lab formulations and marketed formulation were found similar. This was useful for the identification and characterization of Avipattikar churna. It has less than 8% moisture so it cannot be spoiled by chemical changes or due to microbial contamination; hence the formulation can be stocked for a long period. No foreign matter was found because it was prepared after its removal. From the phytochemical analysis it was obtained that kalimircha, ginger, eliyachi shows lack of tannin while Harde, Bahera, Amla detected tannin. The determination of ash values reveals that total ash value of lab formulations were less than the marketed formulation which shows the presence of higher quantity of inorganic constituent in marketed formulation. The acid insoluble ash value indicates small amount of inorganic impurity like silica. From the extractive value, it shows that water soluble extractive value of both formulations was greater than alcohol soluble extractive value which reveals that the better solvent for extraction was water than alcohol. The rheological evaluation indicates the good flow property for all formulations. The standards established in this work can be used for quality evaluation and development of their formulation's quality as reference by Ayurvedic manufactures.

### Pharmacological activity

The review on in-vitro, in-vivo and clinical researches of Avipattikar churna has been published. The anti-secretory and anti-ulcerogenic evaluation of churna was carried out by **Gyawali S. et.al.** with that of ranitidine in Sprague Dawley male rats by pylorus ligation model. In this study, gastric irritancy index and the curative ratio were calculated with the measurement of number of ulcers and their lengths. At the dose of 500 mg/kg, the anti-ulcerogenic and anti-secretory activities of churna were similar to those of ranitidine. Hence the churna was observed a good therapeutic effect. <sup>[34]</sup> **Aswatha Ram et.al.** have reported the gastroprotective action of Avipattikar churna. This study was performed in Albino Wister rat with dose of 500mg/kg by pretreatment model. In reducing the ulcer number and gastric irritancy the effect of churna was found to be slightly lower than that of standard drug. It concludes that Avipattikar churna possess marked gastro-protection. <sup>[35]</sup> The pylorus ligation model and ethanol induced gastric mucosal damage model was taken by **Zaveri M** and **Patel et.al.** that confirms the churna have mucoprotective effect which was assessed by parameters such as mucous content and mucous activity. <sup>[36]</sup> The antioxidant activity in aqueous extract and methanolic extract has been done which reveals their activity due to antioxidant ingredients of avipattikar churna. <sup>[37]</sup> Clinical study on amla pitta was also done. <sup>[38]</sup> **Mahanja MP** and **Hendre SM** confirm its potential for gastric disorders by assessing on gastric specific biomarkers (pepsinogen-1,

pepsinogen-2, gastrin-17, IgG for H. Pylori).<sup>[39]</sup> The comparative clinical study with other formulation ie Patoladi kwath,<sup>[40,41]</sup> and the additive effect study of Avipattikar churna with Sutasekhar Rasa were also carried out.

The success of Ayurvedic treatment in Urdhwaga Amlapitta was carried out by **Saxena Vartika et.al.** It shows that they were found to be well tolerated, safe, and tolerable to the patient.<sup>[42]</sup> In combination with Shankha Bhasma, Prawal Panchamrita Rasa, Kapardhika Bhasma, Mandura Bhasma, Vachadi Churna and Dhatri Lauha etc were used to amplify the action of Avipattikar Churna.<sup>[43,44]</sup>

#### **Avipattikar churna and Kamdudha Ras**

This work explains the probable mode of action of Avipattikar churna and Kamdudha ras in the management of Amlapitta. The combinations of both are rich in Tikta, Katu and Madhur ras dominant drugs, thus they helps to combat etiopathogenesis of amlapitta. It relieves people from the tentacles of the disease. It reveals that proper following of tryoupstambha is very important which can be done by avoiding hurry, worry, curry and by involving tikta ras dominant vegetables, madhur ras dominant fruits in their diet. At least 6-7 hrs of proper sleep should be taken for a healthy and graceful body by avoiding stress and anxiety.<sup>[45]</sup>

#### **Discussion:-**

This work has been focused on traditional polyherbal medicines, Avipathy churnam and Avipattikar churnam. Avipathy churnam includes 10 spices whereas Avipattikara churnam includes 12 spices, salt and sugar. Studies related to the formulation shows that, they were standardized through various physical and chemical tests and evaluated for organoleptic, physicochemical tests and phytochemical tests. The total ash values, extractive values, moisture content were found to be within the acceptable limits. Phytochemical screening studies were also carried out, which shows the presence of secondary metabolites. The flow ability of formulation was assessed by their physical characteristics. Concentrations of different trace elements and heavy metals were estimated. Trace elements such as copper plays a key role in metabolism of iron, calcium function as a constituent of bone and teeth, magnesium and zinc function as an enzyme cofactor. Hence, the formulations containing greater quantity of trace elements are helpful in supporting different functions of human body. The presence of heavy metals such as cadmium, lead, arsenic can cause serious diseases if they are above WHO permissible limit. Based on the results of HPTLC profile, the method can be used for quantitative analysis of marker compound piperine in the raw materials as well as formulations.

The pharmacological activities were carried out for both the formulations. The result shows that Avipathy churnam possess laxative, antiulcer activity and used for hypothyroidism with Chitrakadi vati. The Avipattikar churnam possess anti-oxidant activity, gastroprotective, anti- secretory and anti-ulcerogenic activity. In curing the hyperacidity of multiple etiologies the contents of the formulations are found effective.

#### **Conclusion:-**

For the assessment of quality of drugs on basis of concentration of their active principles, standardization is an essential factor. Both formulations are the oldest herbal formulation for gastric disorders. It was concluded that, phytochemical, pharmacognostical, analytical and pharmacological studies were reported for Avipattikar churna. But in case of Avipathy churnam, still more studies has to be carried out in order to prove quality, safety and efficacy.

#### **Acknowledgement:-**

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#### **Conflict Of Interest**

None.

#### **Abbreviations**

**HPLC:** High Performance Liquid Chromatography, **H-pylori:** Helicobacter pylori, **ATP:** Adenosine Tri Phosphate, **R<sub>f</sub>:** Retention factor, **BMI:** Body Mass Index, **TSH:** Thyroid Stimulating Hormone, **API:** Ayurvedic Pharmacopoeia of India, **WHO:** World Health Organization, **AOAC:** Association of Official Agricultural Chemist.

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