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STANDARDIZATION AND PHARMACOLOGICAL SCREENING OF MARKETED DIGESTIVE CHEWABLE TABLET

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Abstract

The most important challenges faced by herbal formulations arise because of their lack of complete evaluation. Evaluation is necessary to ensure quality and purity of the herbal products. In present study for evaluation of Hazmakar digestive tablet various parameters were tested. These parameters for raw materials include Organoleptic, Physicochemical, Preliminary phytochemical screening and Physical parameters. Quality control parameters for chewable tablet like description, hardness, friability, weight variation, HPTLC fingerprinting & estimation, heavy metal analysis, microbial analysis, acid secretion activity, gastric motility activity and anti-oxidant activity were carried out. Hazmakar digestive tablet consists of four ingredients which are powders of *Piper nigrum* Linn. (Fruit), *Zingiber officinale* Rosc. (Rhizome), *Piper longum* Linn. (Fruit) and *Cuminum cyminum* L. (Fruit) are used to elevate digestive process, give relief from gas troubles and indigestion. Results point out that all raw materials of Hazmakar digestive tablet has passed through all organoleptic, physicochemical, preliminary phytochemical screening and physical parameters.

All three randomly chosen batches of tablets were uniform in description, hardness, friability and weight variation. Estimation of piperine using marker compound has shown presence of different concentration in raw materials and Hazmakar digestive tablet. Heavy metal and microbial contamination was below the detection limit. Acid secretion and gastric motility study revealed that Hazmakar digestive tablet has acid secretion and gastric motility activity in dose dependent manner. Anti-oxidant activity was given by Hazmakar digestive tablet with DPPH and H_2O_2 model. Acid secretion, gastric motility and anti-oxidant activity of Hazmakar digestive tablet may be due to presence of phytoconstituents like alkaloids, flavanoids, phenolics, tannin and carbohydrates.

INTRODUCTION

These days world is witnessing medicine going back to nature – a shift in global trend from synthetic to natural medicine. Medicinal herbs have been known for centuries and are highly valued all over the world as a rich source of therapeutic agents for prevention of diseases and ailments. India is perhaps the largest producer of medicinal herbs and is rightfully called the "Botanical Garden of the World". India also has a very unique position in the world, where a number of recognized indigenous systems of medicine viz. Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are practiced even today for health maintenance.[1]

Herb has various meanings, but in simple terms, it refers to "crude drugs of vegetable origin utilized for the treatment of diseases states, often of a chronic nature, or to attain or maintain a condition of improved health". Herbal preparations called "Phytopharmaceuticals", "Phytomedicinal" or "Phytomedicine", are preparations made from different parts of herbs or plants. They come into different formulations and dosage forms including tablets, capsules, elixir, powder, extract, tincture, cream and parenteral preparations. A single isolate or active principle derived from plants such as digoxin or reserpine tablets is not considered as herbal medicine.[2]

Herbal medicines are in great demand in the developed as well as developing

countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available. Public, academic and government interest in herbal medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine.[3]

In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition. Therefore, quality control of herbal medicines offers a host of problems. To solve this problem, first and foremost task is the selection of the right kind of plant material which is therapeutically efficacious.[4]

Phytochemistry or natural product chemistry research is the backbone of herbal industry and directly/ indirectly responsible for both failure and success of herbal drugs. For promoting the use of herbals in modern medicine, phytochemistry should be envisaged for (1) Isolation, purification and characterization of new phytoconstituents. (2) Use of newly isolated phytoconstituents as "lead" compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity. (3) Conservation of lead phytoconstituents into medicinally important drugs.[5]

The process of evaluating the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observation is called standardization.[6]

Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principle. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous.

Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical location. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation of its quality, safety and efficacy.[7]

Indigestion is a term that describes a feeling of fullness or discomfort in the upper abdomen. Signs of indigestion may be vague but can also include belching, heartburn, bloating, and nausea. Also called dyspepsia (and non-acid dyspepsia), it is a common symptom caused by many conditions and is not a disease. Some investigators suggest heartburn and indigestion are closely related, others separate these two conditions.

In indigenous traditional systems of medicine, the drugs are primarily dispensed as water decoction. Medicinal plant parts must be authentic and free from harmful materials like pesticides, heavy metals, microbial and radioactive contaminations. The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints.

Since ancient times a number of herbal medicines have been used in the treatment of indigestion and there is increasing demand by patients of the natural products with Digestive activity. Hazmakar digestive tablet consists of four ingredients viz., powder of *Piper nigrum* Linn.(Fruit), *Zingiber officinale* Rosc.(Rhizome), *Piper longum* Linn.(Fruit) and *Cuminum cyminum* L.(Fruit). Literature survey of all these plant revealed that they all have reported to be useful as digestive and antioxidant, but yet no scientific reports are available for this formulation. Hence this project was undertaken to check the quality, purity, efficacy of Hazmakar digestive tablet, it was decided to standardize, and to carry out pharmacological screening with suitable animal models.

MATERIALS & METHODS

All raw materials in powder and finished product of Hazmakar digestive tablet were procured from Mehta Unani Pharmacy & Co. Pvt. Ltd., Rajkot. Here part of plants used in the Hazmakar digestive tablet were fruits of *Piper nigrum* Linn., *Piper longum* Linn. and *Cuminum cyminum* L. and rhizome of the *Zingiber officinale* Rosc.. All plant were identified and authenticated by DR. M. S. Jangid, Associate professor, Botany Department, Sir P. T. Science College, Modasa, and a voucher specimens of the same were deposited in the Department of Pharmacognosy, Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa. All the reagents were of analytical grade and instruments were calibrated and facilitated by Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa.

METHODOLOGY

COLLECTION AND AUTHENTICATION OF RAW MATERIAL AND FINISHED PRODUCT

Quality control parameters for raw material

Organoleptic parameter

Physicochemical parameters

a) Loss on drying

[Importance: because raw materials with high moisture are prone to microbial contamination and chemical degradation such as hydrolysis]

b) Determination of ash

- Total ash
- Acid insoluble ash
- Water soluble ash

[Importance: Ash value is material remaining after incineration, gives idea about total mineral content.]

c) Determination of extractive matter

- Alcohol soluble extractive
- Water soluble extractive

[Importance: Helps in selecting suitable solvent system for extraction and determines nature of active ingredients present in raw material.]

Determination of Physical Parameters

a) pH

b) Bitterness value

c) Foaming index

Preliminary Phytochemical Screening

Determination of total tannin content

Quality control parameters for Hazmakar digestive tablet

a) Description
b) Thickness & Diameter
c) Hardness
d) Friability
e) Weight variation

TLC study of raw material and Hazmakar digestive tablet[8]

Solvent system for TLC study and HPTLC study is given in table 1 and TLC study is required for the conformation of mobile phase for HPTLC.

Estimation or fingerprinting of raw material and Hazmakar digestive tablet by HPTLC

HPTLC is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps; stationary phase, mobile phase, development technique and detection. The HPTLC was carried out using a Hemilton 100µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin through chamber, Camag TLC Scanner-3, WINCAT integration software, 0.2mm thick Aluminium sheet precoated with Silica Gel 60F254 (Merck),. HPTLC finger printing technique is useful to identify and check the purity of raw herbal extracts as well as finished product. Hence forth it is very useful tool in standardizing process of raw herbal extracts and finished products.

Steps involved in HPTLC analysis:

Selection of plate and adsorbent: Precoated Aluminium plates with Silica Gel 60F254 (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness was taken 5min prior to chromatography.

Sample solution: Accurately weighed 50mg of methanol extract of *Piper longum* powder was dissolved in methanol and volume was made up to 10ml in volumetric flask. Similarly sample solutions were prepared from powdered ingredients of Hazmakar digestive tablet and were used for HPTLC estimation or fingerprinting.

Application of sample: Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The automatic application devices are preferable. The advance automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm width with different concentration of *Piper longum* methanolic

solution viz. 10, 20, 30, 40, 50µg/ml.

Development: The plate was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the solvent for 60 min (temperature 25.2 °C, relative humidity 40%). The development distance was 8 cm. Subsequently scanning was done. The mobile phase or solvent system for all the ingredient as well as Hazmakar digestive tablet which is given in the table 1.

Detection: The plate was scanned at UV 366 nm and 254 nm using CAMAG TLC Scanner-3 and LINOMAT-V. R_f value of each compound which were separated on plate and data of peak area of each band was recorded.

Heavy metals analysis of Hazmakar digestive tablet

a) Lead
b) Arsenic
c) Mercury
d) Cadmium

[Importance: WHO and pharmacopoeias have decided limits for heavy metals in pharmaceutical preparations. Higher metallic contamination makes finished product unsuitable for use.]

Microbial analysis of Hazmakar digestive tablet

a) Total plate count
b) Presence of *Escherichia coli*
c) Presence of *Staphylococcus aureus*
d) Presence of *Pseudomonas aeruginosa*

[Importance: Checking microbial status of the finished product is obviously important. Microbial contamination higher than specified values is not allowed in pharmaceutical preparations.]

PHARMACOLOGICAL SCREENING OF HAZMAKAR DIGESTIVE TABLET

Determination of acid secretion by Hazmakar digestive tablet using Pylorus ligation model in Wistar albino rats

The animals were fasted for overnight, before Pylorus ligation but free access to water. Under light ether anesthesia, the abdomen was opened by midline incision process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. Test drug solution was administered before Pylorus ligation. The stomach was placed back carefully and the abdominal wall was closed with sutures. Animals were sacrificed 4 h after Pylorus ligation. The stomachs were

isolated and the content of the stomach were collected and centrifuged. The volume of the gastric juice was measured and this was used for estimation of pH, free acidity and total acidity.[9]

Determination of pH: An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH-meter.

Determination of total acidity: An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity was expressed as mEq/L by the following Formula:

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{N} \times 100\ \text{mEq/L}}{0.1}$$

Determination of free acidity: Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

Statistical analysis: Results are presented as Mean±SEM of six animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett test using graph pad prism software. The significance difference if any among the groups at $p < 0.05$ was considered statistically significant.

Determination of intestinal transit time by Hazmakar digestive tablet in male Wistar rats using charcoal[10]

Charcoal suspension is given orally, with the drug to be tested, after an overnight fast. Subjects are killed 20–30 min after treatment, and any charcoal in the stomach is noted along with the distance travelled through the GI tract. Overnight fasting affects the responsiveness of the GI tract to lumen contents and, moreover, drug–feed interactions could result in the production of peptides that cause changes in transit time. The intestinal transit of charcoal suspension was determined by modified janseen's method. The formula is as follows:

$$\% \text{ Transit time} = \frac{\text{Distance travelled by charcoal suspension} \times 100}{\text{Total length of small intestine}}$$

Statistical analysis: Results are presented as Mean±SEM of six animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett test using graph pad prism software. The significance difference if any among the groups at $p < 0.05$ was considered statistically significant.

Anti oxidant activity of Hazmakar digestive tablet using

DPPH radical scavenging model[11,12]

The antioxidant reacts with stable free radical, DPPH and converts it to 1,1-Diphenyl-2-Picryl Hydrazine. The free radical scavenging activity of the product extract, based on the scavenging activity of the stable (DPPH) free radical was determined.

Scavenging of Hydrogen peroxide model[13]

The ability of extract of product to scavenge hydrogen peroxide was determined.

RESULTS AND DISCUSSION

Quality control parameters for raw material of Hazmakar digestive tablet

Organoleptic parameters

All the powders used in Hazmakar digestive tablet were passed through 80#. Description of powder of all plants parts used in the formulation of Hazmakar tablet is given in table 2

Results indicated that all of ingredients of Hazmakar digestive tablet was easily distinguished by their specific colour, odour and taste.

Physicochemical parameters

Loss on drying

Loss on drying in powder of *Zingiber officinale* was highest and lowest *Cuminum cyminum* among the all ingredients. Loss on drying of all the ingredients of Hazmakar digestive tablet were less than 10 % w/w, so there is less chances of microbial growth.

Ash value

Total ash, Acid insoluble ash and Water soluble ash of powders of *Piper nigrum, Zingiber officinale, Piper longum* and *Cuminum cyminum* were not more than

limit. Results reveal that the value of total ash, acid insoluble ash value and water soluble ash value in all four plant powder was less than the limit prescribed in authentic book, indicated that the all the plant powder were free from impurities.

Extractive value

Water soluble extractive value of all the ingredients of Hazmakar digestive tablet was higher than alcohol soluble extractive value. It indicated that Hazmakar digestive tablet has good water solubility.

Physical parameter

pH

pH value of powder of *Piper longum* was higher and powder of *Zingiber officinale* was lower. pH value of all the ingredients of Hazmakar digestive tablet are in neutral range.

Bitterness value

Bitterness value of powder of *Piper nigrum* was 0.89, powder of *Zingiber officinale* was 0.98, powder of *Piper longum* was 1.48 and powder of *Cuminum cyminum* was 0.56. Results indicated that all the ingredients have shown lowest bitterness value and helps in indentifying the quality of Hazmakar digestive tablet.

Foaming index

Foaming index of powders of *Piper nigrum*, *Zingiber officinale*, *Piper longum* and *Cuminum cyminum* were less than 100. Foaming index of the ingredients is of important for the identifying saponin containing sample. So, here none of ingredients of Hazmakar digestive tablet contains Saponin.

Preliminary phytochemical screening

Alkaloids were present in powders of *Piper nigrum* and *Piper longum,* and absent in all other ingredients of Hazmakar digestive tablet. Flavanoids were present in powders of *Zingiber officinale* and *Cuminum cyminum.* Tannins were present in powders of *Piper nigrum* and *Cuminum cyminum* while absent in all others. Phenolic content were present only in powder *Zingiber officinale* and *Cuminum cyminum*. Carbohydrates and amino acids were present in all the ingredients of Hazmakar digestive tablet. Glycosides, steroids and terpenoids were absent in all ingredients of Hazmakar digestive tablet.

Total tannin content

Tannin content of powder of *Piper nigrum* was 1.039% and powder of *Cuminum cyminum* was 0.519%. Results shown that

ingredient of Hazmakar digestive tablet contain minor amount of tannin.

Quality control parameters for finished product

All the three batches have similarity in colour, odour, taste, thickness and diameter. Hardness of all three batches was more than 4 kg/cm^2. Weight variation of all three batches of Hazmakar digestive tablet was less than 5% and friability was less than 1%. Result had shown uniformity among all three batches of Hazmakar digestive tablet.

ESTIMATION OR FINGERPRINTING OF RAW MATERIAL AND HAZMAKAR DIGESTIVE TABLET BY HPTLC

Estimation of Piperine in *Piper nigrum* and *Piper longum*

Calibration of piperine

Peak areas of Piperine for (10-50µg/ml) concentration were recorded. Calibration curve was prepared by plotting peak areas of Piperine against concentration. The results of linearity range and correlation coefficient within the concentration (10µg/ml-50µg/ml) range indicated, there was good correlation between peak area and the corresponding concentration of Piperine as shown in Fig. 1. The best fitting line equation was y = 64.59x + 29.41.

Determination of piperine in Hazmakar digestive tablet, *Piper longum* and *Piper nigrum*

Silica gel TLC plate as stationary phase and Toluene: Ethyl acetate (70:30) as mobile phase gives good separation of Piperine at R_f = 0.57. The HPTLC plate photograph of Standard Piperine, Hazmakar digestive tablet, *Piper nigrum* and *Piper longum* is shown in Fig 4. Correlation coefficient 0.992 indicates good linearity between concentration and peak area.

The concentrations of Piperine in the methanolic extract of powder of *Piper longum, Piper nigrum* and Hazmakar digestive tablet by the proposed HPTLC method were found to 2.90µg/ml, 2.47µg/ml and 0.312µg/ml respectively. The identity of the Piperine band in the sample extract solution was confirmed by overlaying /superimposing the UV absorption spectrum of the sample with that from the reference standard of Piperine, using the Camag TLC scanner 3. Results shows that *Piper longum* and *Piper nigrum,* raw materials of Hazmakar

digestive tablet and 'Hazmakar digestive tablet' both contain piperine indicated presence of *Piper longum* and *Piper nigrum* in Hazmakar digestive tablet.

Fingerprinting of *Zingiber officinale* Rosc.

Results indicated that Chromatograph of Hazmakar digestive tablet and *Zingiber officinale* had shown presence of peaks having R_f value of 0.14 and 0.25 similar in both, which indicated same compounds present in both i.e. Hazmakar digestive tablet and *Zingiber officinale*.

Fingerprinting of *Cuminum cyminum* L.

Results indicated that HPTLC chromatograph of Hazmakar digestive tablet and *Cuminum cyminum* had shown presence of peaks having R_f value of 0.08, 0.47 and 0.55 similar in both, which indicated same compounds present in both i.e. Hazmakar digestive tablet and *Cuminum cyminum*.

Heavy metal analysis of Hazmakar digestive tablet

Results indicated that concentrations of Arsenic, Mercury and Cadmium in 'Hazmakar digestive tablet' were not in detectable amount. Concentration of Lead in 'Hazmakar digestive tablet' was below standard limit. Results indicated that heavy metal content in Hazmakar digestive tablet was less than the prescribed limit.

*Test was performed by Gujarat laboratory, Madhavpura market, Ahmedabad.

Microbial analysis of Hazmakar digestive tablet

Total plate count of Hazmakar digestive tablet was 380cfu/gm. It was below standard limit 1×10^5 cfu/gm. *Escherichia coli, staphylococcus aureus* and *Pseudomonas aeruginosa* were not found in detectable amount in the formulation. Results revealed that Microbial contamination in Hazmakar digestive tablet was below the standard limit.

*Test was performed by Gujarat laboratory, Madhavpura market, Ahmedabad.

PHARMACOLOGICAL SCREENING OF HAZMAKAR DIGESTIVE TABLET

Determination of acid secretion by Hazmakar digestive tablet using Pylorus ligation model in Wistar albino rats

Acid secretion activity in Wistar albino rat was determined using Pylorus ligation model. Effect on normal group (distilled water), group 1 (250mg/kg of Hazmakar

digestive tablet) and group 2 (500mg/kg of Hazmakar digestive tablet) were recorded. Result of acid secretion activity is shown in table 17 and graphical presentation is given in Fig. 6.

Results indicated in Mean ± SEM, where n=6, statistically analysis by one-way analysis of variance (ANOVA) followed by Dennett's multiple comparison test. All groups having p (< 0.05) value was significant.

Normal group shown 4.08 ± 0.047ml gastric secretion, 4.23 ± 0.076 pH, 83.42 ± 1.565mEq/L free acidity and 103.71 ± 2.060mEq/L total acidity. Group 1 shown 5.25 ± 0.042ml gastric secretion, 3.54 ± 0.108 pH, 107.95 ± 1.120mEq/L free acidity and 120.30 ± 1.358mEq/L total acidity. Group 2 shown 5.91 ± 0.047ml gastric secretion, 3.34 ± 0.043 pH, 116.01 ± 1.888mEq/L free acidity and 130.38 ± 1.587mEq/L total acidity. Results indicated that Hazmakar digestive tablet exhibit acid secretion activity in dose dependent manner.

Results indicated that Hazmakar digestive tablet exhibit acid secretion activity in dose dependent manner. Acid secretion activity of Hazmakar digestive tablet may be due to presence of phytoconstituents like alkaloids, phenolic and volatile compounds.

Determination of intestinal transit time by Hazmakar digestive tablet in male Wistar rats using charcoal

Intestinal transit time in male Wistar rats was determined using charcoal. Effect on normal group and group 1 (500 mg/kg of Hazmakar digestive tablet) were recorded. Result of determination of intestinal transit time is shown in table 18 and graphical presentation is given in Fig. 7.

Results indicated in Mean ± SEM, where n=6, statistically analysis by one-way analysis of variance (ANOVA) followed by Dennett's multiple comparison test. All groups having p (<0.05) value was significant.

Normal group shown 72.33 ± 0.701% transit time, while Group 1 (Hazmakar digestive tablet: 500mg/kg) shown 89.68 ± 0.554% transit time. Results indicated that Hazmakar digestive tablet increase intestinal transit time in rat.

Results indicated that Hazmakar digestive tablet increase intestinal transit time.

Increase in gastric motility by Hazmakar digestive tablet may be due to presence of phytoconstituents like phenolic content like gingerol and volatile compounds.

Anti oxidant activity of Hazmakar digestive tablet

The antioxidant activity of the alcoholic extract of the formulation was carried out by *in vitro* antioxidant models using ascorbic acid as standard antioxidant.

DPPH radical scavenging model

Antioxidant activity of Hazmakar digestive tablet was carried out using DPPH model comparing with ascorbic acid as standard antioxidant. Results are given in table 19 and graphical presentation is given in Fig.8.

There was a reduction in the concentration of DPPH radicals due to the scavenging ability by increasing the dose of alcoholic extract of Hazmakar digestive tablet and Ascorbic acid, as a reference standard. Maximum inhibition of DPPH radicals scavenging ability with 100µg/ml of alcoholic extract of Hazmakar digestive tablet and Ascorbic acid was exhibited 21.22% and 96.23% respectively. The IC_{50} values in DPPH radical scavenging model were 45.51µg/ml and 280.57µg/ml for Ascorbic acid and alcoholic extract of Hazmakar digestive tablet respectively.

Scavenging of Hydrogen peroxide model

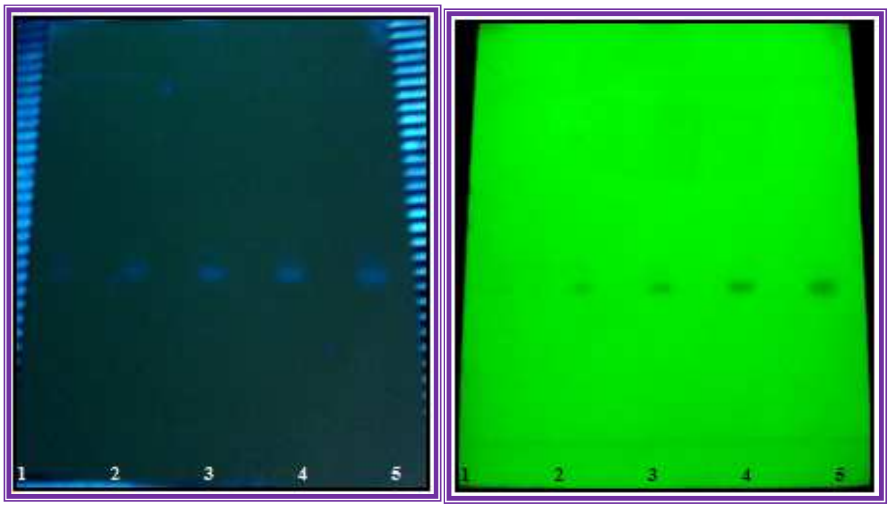
Antioxidant activity of Hazmakar digestive tablet was carried out using hydrogen peroxide model comparing with ascorbic acid as standard antioxidant. Results are given in table 20 and graphical presentation is given in Fig.9.

There was a significant reduction in the concentration of H_2O_2 radicals due to the scavenging ability by increasing the dose of alcoholic extract of Hazmakar digestive tablet and Ascorbic acid, as a reference standard. Maximum inhibition of H_2O_2 radical scavenging ability with 100µg/ml of alcoholic extract of Hazmakar digestive tablet and Ascorbic acid was exhibited 38.91% and 73.34% respectively. The IC_{50} values in DPPH radical scavenging model were 60.49µg/ml and 132.09µg/ml for Ascorbic acid and alcoholic extract of Hazmakar digestive tablet respectively.

CONCLUSION

Data suggested that Hazmakar digestive tablet and its ingredients were consistent with various quality and purity parameters such as organoleptic parameters,

physicochemical parameters, HPTLC analysis, heavy metal analysis and microbial analysis. Along with increase in gastric acid secretion and gastric motility, Hazmakar digestive tablet also gave antioxidant activity.



A- Under UV 366 nm B- Under 254 nm

[Track-1: 10 µg/ml of Standard Piperine. Track-2: 20 µg/ml of Standard Piperine. Track-3: 30 µg/ml of Standard Piperine. Track-4: 40 µg/ml of Standard Piperine. Track-5: 50 µg/ml of Standard Piperine.]

Figure 1: HPTLC plate of standard piperine

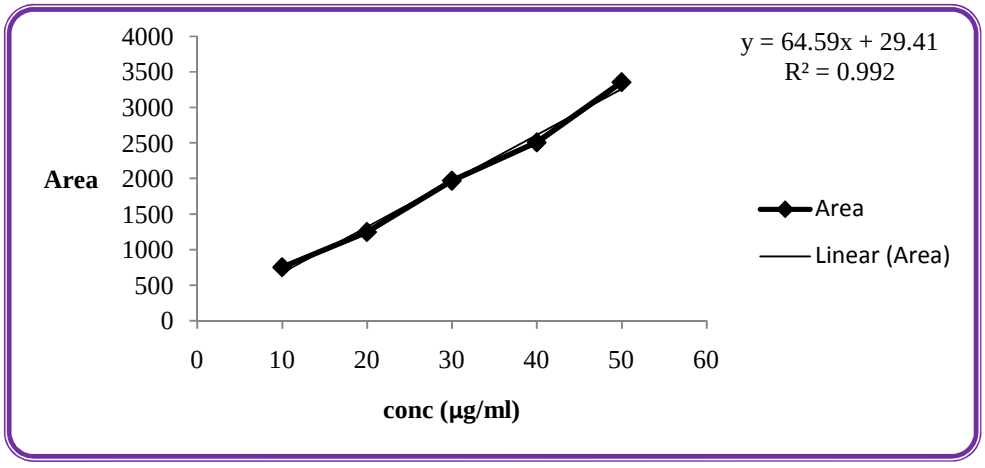
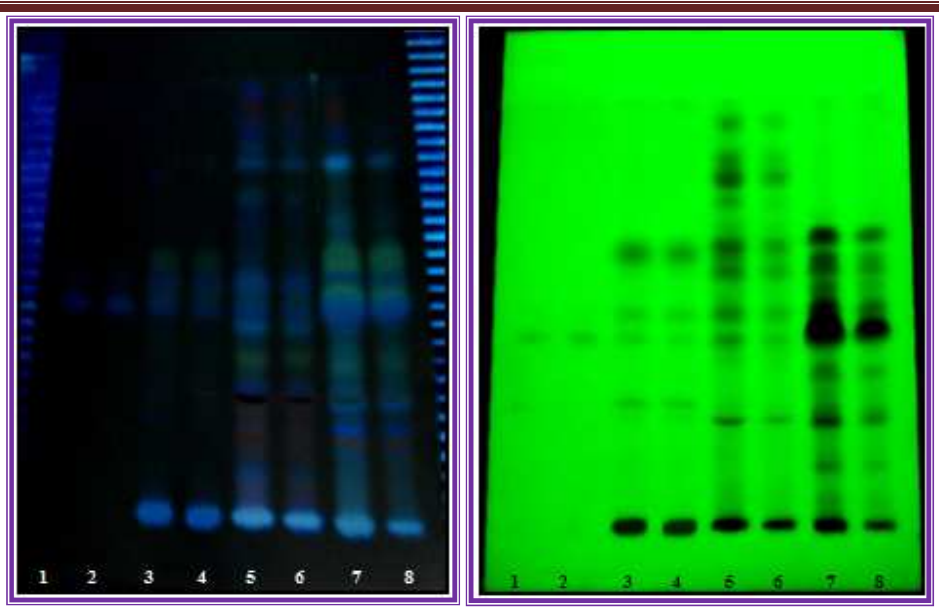


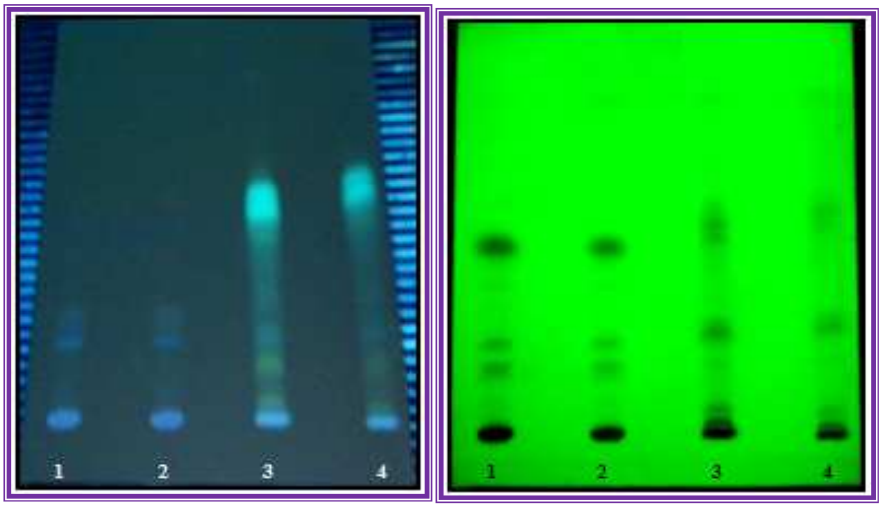
Figure 2: Calibration curve of piperine



A- Under 366nm B-Under 254nm

[Track 1: 10µg/ml std piperine, Track 2: 20µg/ml std piperine, Track 3: 10µg/ml formulation, Track 4: 20µg/ml formulation, Track 5:10 µg/ml *P. longum*, Track 6:20 µg/ml *P. longum,* Track 7: 10µg/ml *P. nigrum*, Track 8: 20µg/ml *P. nigrum*]

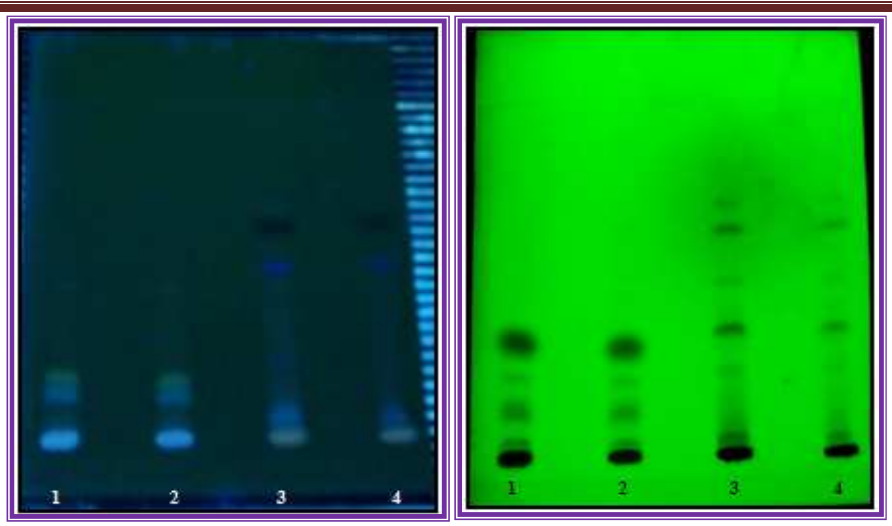
Figure 3: HPTLC plate of Hazmakar digestive tablet, *P. longum and P. nigrum*



A-Under 366nm B-Under 254nm

[Track 1: 10 µg/ml Hazmakar digestive tablet, Track 2: 20 µg/ml Hazmakar digestive tablet, Track 3: 10 µg/ml of *Z. officinale*, Track 4: 20 µg/ml *Z. officinale*]

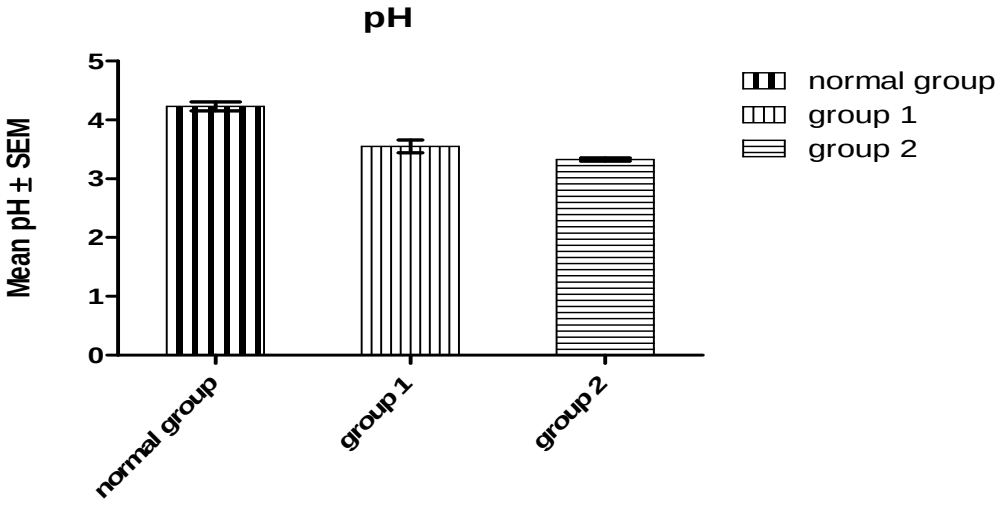
Figure 4: HPTLC plate of Hazmakar digestive tablet and *Zingiber officinale*



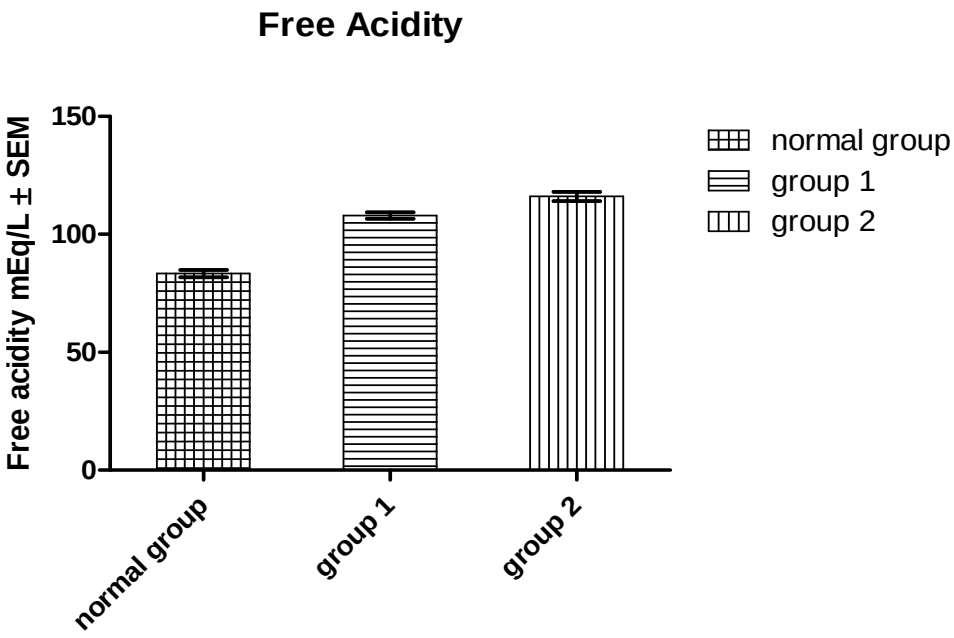
A- Under UV 366 nm B-Under 254nm

[Track 1: 10 µg/ml Hazmakar digestive tablet, Track 2: 20 µg/ml Hazmakar digestive tablet, Track 3: 10 µg/ml of *C. cyminum*, Track 4: 20 µg/ml *C. cyminum*]

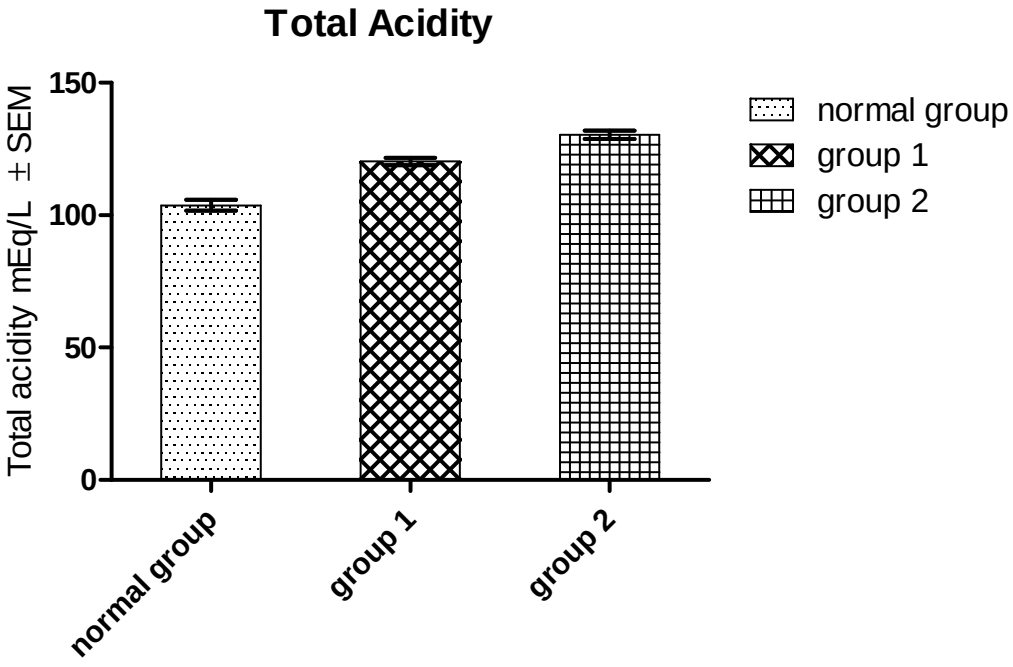
Figure 5: HPTLC plate of Hazmakar digestive tablet and *Cuminum cyminum*



(a) Effect of Hazmakar digestive tablet on pH of gastric content



(b) Effect of Hazmakar digestive tablet on free acidity of gastric content



(c) Effect of Hazmakar digestive tablet on total acidity of gastric content

Figure 6: Graphical presentation of acid secretion activity

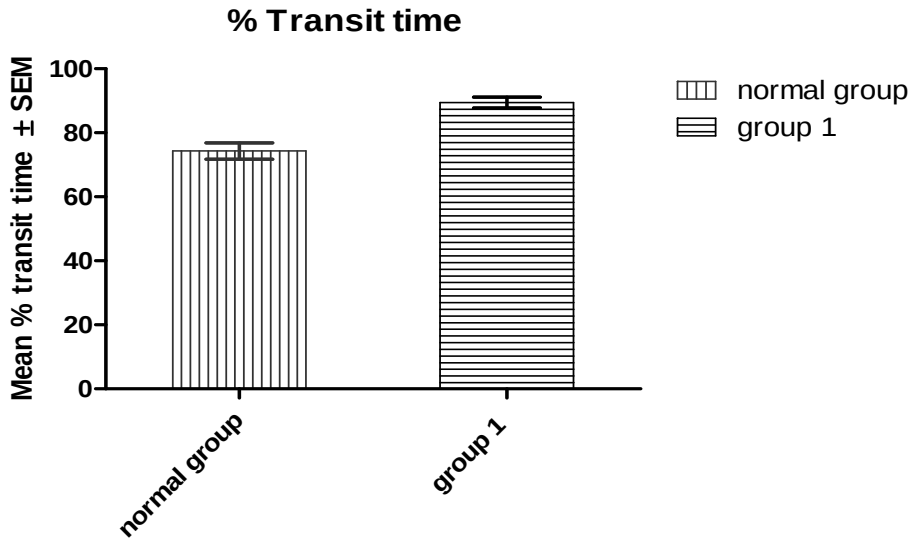


Figure 7: Graphical presentation of determination of Intestinal transit time

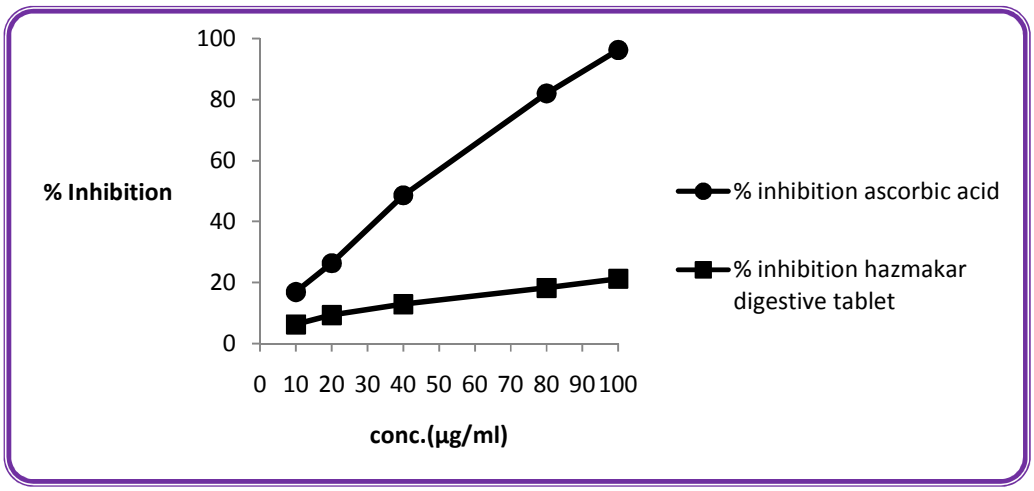


Figure 8: DPPH radical scavenging activity of Hazmakar digestive tablet

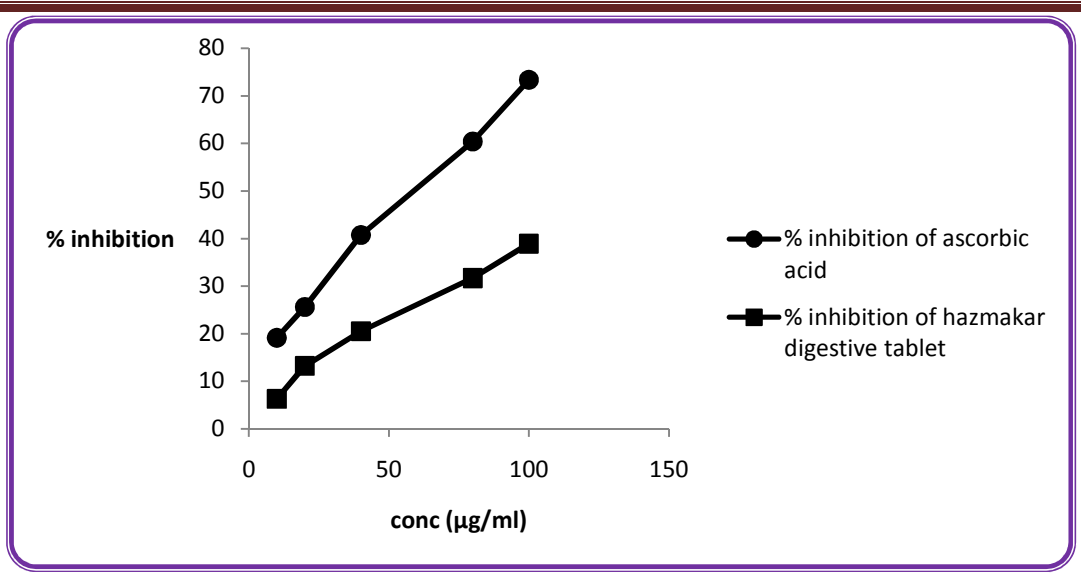


Figure 9: Hydrogen peroxide scavenging activity of Hazmakar digestive tablet

Table 1

Solvent system for raw materials and Hazmakar digestive tablet

Sr. No.	Sample Name	Solvent system
1	*Piper nigrum* Linn. , *Piper longum* Linn. (Piperine)	Toluene : Ethyl Acetate (70:30)
2	*Zingiber officinale* Rosc. (Gingerol)	n-Hexane :Ether (4 : 6)
3	*Cuminum cyminum* L. (Carvone)	Toluene : Ethyl Acetate (90 : 10)

Table 2

Organoleptic properties of 'Hazmakar digestive tablet' ingredients

Sr. No.	Ingredients	Plant Part	Colour	Odour	Taste
1	*Piper nigrum*	Fruits	Grayish black	Aromatic	Pungent
2	*Zingiber officinale*	Rhizomes	Brown	Characteristic	Pungent
3	*Piper longum*	Fruits	Grayish black	Aromatic	Pungent
4	*Cuminum cyminum*	Fruits	Buff	Aromatic	Aromatic

Table 3

Loss on drying of 'Hazmakar digestive tablet' ingredients

Sr. No.	Ingredients	Loss on drying (%w/w)
1	*Piper nigrum*	4.96 ± 0.09
2	*Zingiber officinale*	8.46 ± 0.088
3	*Piper longum*	6 ± 0.11
4	*Cuminum cyminum*	2.46 ± 0.13

Table 4

Ash value of 'Hazmakar digestive tablet' ingredients

Sr. No.	Ingredients	Total Ash (%w/w)	Acid insoluble Ash (%w/w)	Water Soluble Ash (%w/w)
1	*Piper nigrum*	5.7 ± 0.057	0.67 ± 0.0008	0.25 ± 0.011
2	*Zingiber officinale*	6.44 ± 0.023	1.55 ± 0.005	0.19 ± 0.0088
3	*Piper longum*	9.34 ± 0.005	1.05 ± 0.014	5.81 ± 0.0208
4	*Cuminum cyminum*	7.23 ± 0.066	5.33 ± 0.015	0.98 ± 0.0085

Table 5

Extractive value of 'Hazmakar digestive tablet' ingredients

Sr. No.	Name of Ingredient	Water soluble extractive (% w/w)	Alcohol soluble extractive (% w/w)
1	*Piper nigrum*	11.50 ± 0.058	10.23 ± 0.120
2	*Zingiber officinale*	20.50 ± 0.058	11.20 ± 0.057
3	*Piper longum*	26.16 ± 0.088	10.66 ± 0.088
4	*Cuminum cyminum*	24.00 ± 0.179	14.50 ± 0.0577

Table 6

pH value of 'Hazmakar digestive tablet' ingredients

Sr. No.	Ingredient	pH (1% w/v sol.)
1	*Piper nigrum*	6.46
2	*Zingiber officinale*	6.04
3	*Piper longum*	6.61
4	*Cuminum cyminum*	6.11

Table 7

Bitterness value of 'Hazmakar digestive tablet' ingredients

Sr. No.	Name of Ingredient	Bitterness value (Units/gm)
1	*Piper nigrum*	0.89
2	*Zingiber officinale*	0.98
3	*Piper longum*	1.48
4	*Cuminum cyminum*	0.56

Table 8

Foaming index of 'Hazmakar digestive tablet' ingredients

Sr. No.	Name of Ingredient	Foaming Index
1	*Piper nigrum*	>100
2	*Zingiber officinale*	>100
3	*Piper longum*	>100
4	*Cuminum cyminum*	>100

Table 9

Preliminary phytochemical screening of ingredients of 'Hazmakar digestive tablet'

Sr. No.	Test	*P. nigrum*	*Z. officinale*	*P. longum*	*C. cyminum*
1	Alkaloid	+	-	+	-
2	Glycoside	-	-	-	-
3	Flavanoid	-	+	-	+
3	Tannin	+	-	-	+
4	Phenolic	-	+	-	+
5	Steroids/ Terpenoids	-	-	-	-
6	Carbohydrates	+	+	+	+
7	Amino acid	+	+	+	+

(+): presence and (-): absent

Table 10

Total tannin content of 'Hazmakar digestive tablet' ingredients

Sr. No.	Name of Ingredient	Tannic acid %w/w
1	*Piper nigrum*	1.039 %
2	*Cuminum cyminum*	0.519 %

Table 11

Quality control parameters for Hazmakar digestive tablet

Parameter	Observation			Remark
	Batch no. 207	Batch no. 394	Batch no. 435	
Organoleptic parameter				
colour	Yellowish brown	Yellowish brown	Yellowish brown	-----
odour	characteristics	characteristics	characteristics	-----
taste	Astringent	Astringent	Astringent	-----
Thickness & Diameter	11.90mm & 5.42 mm	11.90mm & 5.42 mm	11.90mm & 5.42 mm	-----
Hardness	5.0 kg/cm^2	5.0 kg/cm^2	4.5 kg/cm^2	Pass
Friability	0.046 %	0.050 %	0.048 %	Pass
Weight variation	< 5 %	< 5 %	< 5 %	Pass

Table 12

HPTLC of Hazmakar digestive tablet, *P. longum and P. nigrum*

Track	Start R_f	End R_f	Area	Conc(µg/ml)
Piper longum	0.52	0.59	216.8	2.90
Piper nigrum	0.51	0.57	189.54	2.47
Hazmakar digestive tablet	0.55	0.58	49.58	0.312

Table 13

HPTLC data of Hazmakar digestive tablet and *Zingiber officinale*

Peak	Hazmakar digestive tablet		*Zingiber officinale*	
	Max. R_f	Peak Area	Max. R_f	Peak Area
1	0.07	5089.2	0.08	9160.6
2	0.14	328.4	0.14	1259.9
3	0.18	488.5	0.16	168.0
4	0.25	2696.4	0.25	1553.0
5	0.35	2730.1	0.39	7099.5
6	0.49	530.7		

Table 14

HPTLC data of Hazmakar digestive tablet and *Cuminum cyminum*

Peak	Hazmakar digestive tablet		*Cuminum cyminum*	
	Max. R_f	Peak Area	Max. R_f	Peak Area
1	0.08	5549.6	0.05	10672.1
2	0.13	5089.8	0.08	1866.1
3	0.20	7871.5	0.14	3164.5
4	0.26	4432.3	0.17	847.1
5	0.40	9804.9	0.22	863.9
6	0.47	1393.2	0.28	1250.6
7	0.55	727.3	0.34	1311.7
8			0.41	3933.2
9			0.47	306.9
10			0.50	654.2
11			0.52	482.8
12			0.55	543.2
13			0.57	82.4

Table 15

Heavy metal analysis of 'Hazmakar digestive tablet'*

Sr. No.	Heavy Metal	Result (mg/kg)	Limit(mg/kg)[14]
1	Lead(Pb)	3.18	10
2	Arsenic(As)	Nil	03
3	Mercury(Hg)	Nil	01
4	Cadmium(Cd)	Nil	0.3

Table 16

Microbial analysis of 'Hazmakar digestive tablet'*

Sr. No.	Test name	Result	Limit[14]
1	Total plate count cfu / gm	380 cfu	1×10^5 cfu / gm
2	Presence of *Escherichia coli*	Absent	Absent
3	Presence of *Staphylococcus aureus*	Absent	Absent
4	Presence of *Pseudomonas aeruginosa*	Absent	Absent

Table 17

Acid secretion activity of 'Hazmakar digestive tablet'

Sr. No.	Group	Gastric secretion volume (ml)	pH	Free acidity mEq/L	Total acidity mEq/L
1	Normal group	4.08 ± 0.047	4.23 ± 0.076	83.42 ± 1.565	103.71 ± 2.060
2	Group 1: Hazmakar digestive tablet (250mg/kg)	5.25 ± 0.042	3.54 ± 0.108	107.95 ± 1.120	120.30 ± 1.358
3	Group 2: Hazmakar digestive tablet (500mg/kg)	5.91 ± 0.047	3.34 ± 0.043	116.01 ± 1.888	130.38 ± 1.587

Table 18

Determination intestinal transit time by Hazmakar digestive tablet

Sr. No.	Group	Total length of small intestine (cm)	Distance travelled by charcoal (cm)	%Transit time
1	Normal group	108.83 ± 0.755	78.33 ± 1.149	72.33 ± 0.701
2	Group 1 Hazmakar digestive tablet (500mg/kg)	109.03 ± 0.821	97.78 ± 0.643	89.68 ± 0.554

Table 19

DPPH radical scavenging activity of Hazmakar digestive tablet

Sr. No.	Conc (µg/ml)	Hazmakar digestive tablet	Ascorbic acid
1	IC_{50} value	280.57	45.51
2	Regression equation	Y=0.158x + 5.669	Y=0.886x + 9.678
3	R^2	0.986	0.996

Table 20

Hydrogen peroxide scavenging activity of 'Hazmakar digestive tablet'

Sr. No.	Conc (µg/ml)	Hazmakar digestive tablet	Ascorbic acid
1	IC_{50} value	132.09	60.49
2	Regression equation	Y=0.340x + 5.088	Y=0.589x + 14.37
3	R^2	0.985	0.994

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