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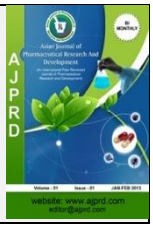

Open Access **Research Article**

Standardization of marketed formulation Kipen Tablet for analgesic and anti-inflammatory potential

Md. Sarfuddin Ansari, Deepak Kumar Basedia, B.K. Dubey

Technocrats Institute of Technology-Pharmacy, Bhopal, MP, India

A B S T R A C T

The primary objective of the current investigation was to standardize the formulation Kipen Tablet marketed as patented and proprietary medicine for treatment of various conditions of Pitta, according to the WHO guidelines. The procured tablets of Kipen were Light green in color, with no odor and a bitter taste. Kipen exhibited 8.1% total ash with 2.35% acid insoluble ash and 4.95% water soluble ash. The water soluble and alcohol soluble extractives were 1.36% and 1.3% respectively. The results of preliminary qualitative phytochemical screening revealed that all the classes of phytochemicals were present in Kipen. TLC analysis of Kipen was done using Curcumin as the marker using ethyleacetate: n-hexane (3:7) as the solvent system. Curcumin appeared at Rf value of 0.56 on the TLC plate. The quantitation of the curcumin was done by HPLC method and it was found that Kipen contained 3.75 mg curcumin per 250 mg of Kipen (1.5 %). The anti-inflammatory activity was evaluated using carrageenan induced rat paw edema method and it was seen that Kipen was able to inhibit about 51% edema formation in rat paws. The analgesic action of Kipen was evaluated using tail flick method and the response time pain stimulus (thermal) was observed. The highest reaction time for Kipen was 6.26 ± 0.06.15 ± 0.242 sec at 30 min post administration while it was 3.46 ± 0.095 sec and 6.22 ± 0.171 sec for vehicle treated and Ibuprofen treated group respectively at the same time duration.

Key words: Kipen tablet, anti-inflammatory, standardization, curcumin, analgesic, ash value

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***Address for Correspondence:**
Md. Sarfuddin Ansari, Technocrats Institute of Technology-Pharmacy, Bhopal, MP, India

INTRODUCTION

Ayurveda is being practiced throughout India since ages and has been vital part of the Indian Medicinal System. Herbal medicinal plants are an essential segment of research advancements in the pharmaceutical business. The World Health Organization (WHO) had given a short convention for standardization of homegrown medications. Standardization is a guarantee that each item that has been used and recommended is of the quality and does the purpose it is meant for.

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. The World Health Organization (WHO) encourages, recommends and promotes traditional/ herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them[2]. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies[3]. As standardization of herbal products has become the need of the hour, the literature reports several articles related to the same[4-17].

The primary objective of this work was to standardize an online promoted herbal tablet indicated for stomach pain, headache, body pain and several other conditions detailing for quality and viability. The standardization of this formulation with respect to its physicochemical properties, organoleptic properties, marker quantitation and its analgesic and anti-inflammatory action was the goal of the present investigation.

MATERIAL AND METHODS

Materials

Kipen Tablet (KT) was purchased from the online store of Tanvi herbal collection (India) Pvt Ltd. Curcumin was used

as the marker compound and was purchased from Oxford Fine Chemicals Pvt Ltd. All chemicals and reagents used were for AR grade and purchased from Oxford Fine Chemicals, Mumbai. Experimental animal were procured from approved local breeders.

Methods

Collection of marketed product for standardization

The marketed formulation Kipen tablet was purchased from online store of Tanvi Herbals. The material was received in discrete packaging containing 10 tablets of the formulation. The formulation was abbreviated as KT for the study.

Organoleptic Standardization of KT

Organoleptic properties are the aspects of food or other substances as experienced by the senses, including taste, sight, smell, and touch, in cases where dryness, moisture, and stale-fresh factors are to be considered[18].

Determination of Total Ash

2 g of KT was placed in a suitable tared crucible of silica previously ignited and weighed. The powder was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash[19] (W.H.O, 1998).

Acid Insoluble Ash

The ash obtained as above was boiled for 5 min with 25 mL of dilute hydrochloric acid; the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated[20].

Water Soluble Ash

The ash was boiled for 5 min with 25 mL of water; the insoluble matter was collected in an ash less filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 45°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to air-dried drug was calculated[20].

Alcohol Soluble Extractive Value

5 g of KT was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during the first six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air dried drug and is represented as % value[21].

Water Soluble Extractive Value

5 g of KT was macerated with 100ml of chloroform-water in a closed flask for twenty-four hours, shaking frequently during the first six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air dried drug and is represented as % value[21].

Preliminary Phytochemical Screening of KT[22]

Alkaloids

The testing for presence of alkaloids was done by dissolving KT in 5 mL of 1% HCl solution. This solution was then subjected to **Mayer's test** of alkaloids.

Glycosides

The test for presence of glycosides (deoxy sugar) was performed according to **Keller-Killiani's** method.

Saponins

0.1 g of KT was boiled in 1 mL distilled water and filtered. To the filtrate was added 3 ml distilled water, shaken vigorously and heated. The sample was observed for the persistence appearance of foam lasting for at least 15 min was taken as confirmation for the presence of saponins.

Tannins and phenolic compounds

To the sample of KT was added a freshly prepared solution of ferric chloride. Development of blue-green color is taken as indication for the presence of tannins and phenolics.

Alkaline reagent test was performed for confirming the presence of tannins. Test solution of KT is treated with sodium hydroxide solution. The formation of yellow or red colored precipitate is an indication of the presence of tannins in the sample.

Flavonoids

To the test solution of KT, a mixture of zinc dust and concentrated hydrochloric acid was added. If the colour of the solution changes to red, it is taken as a confirmation for the presence of flavonoids.

Proteins and Amino acids

0.1 g of KT was dispersed in water and was boiled with 0.2% solution of ninhydrin. Development of violet colour in the solutions is an indicator for the presence of amino acids and proteins in the sample.

Sterols and Triterpenoids

Libermann-Burchard test was performed to detect the presence of steroids in the formulation. KT was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube. Change in color from violet to blue or green indicates the presence of steroids.

Salkowski test was done to confirm the presence or absence of triterpenoids. KT was dissolved in chloroform and a few drops of concentrated sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time to separate into layers. The formation of greyish colour indicates the presence of terpenes/terpenoids.

TLC analysis of KT and curcumin standard

TLC- method was developed using Precoated TLC Plate (Silica gel 60 F_{254}) for the standardization of KT. Different solvents Toluene, Benzene, Ethyle Acetate, Acetic Acid, Formic Acid, Chloroform, Methanol, Water, Hydrochloric Acid were screened for development of mobile phase. The Visualizing agent such as UV chamber, Iodine, Folins reagent, Methanolic Ferric chloride and Mayer's reagent were used to identify the spots. The optimized mobile was ethyleacetate: n-hexane (3:7).

Quantitative estimation of Curcumin in KT

Curcumin in the KT was quantified by a HPLC method which involved using a C18 column, UV detector and detection wavelength of 425 nm and flow rate of 2.0 mL/min for the mobile phase comprising of acetonitril and 2% v/v acetic acid (40:60, v/v)[23]. The total duration of run was 20 min. Curcumin standard solutions were prepared in various concentrations of 10, 20, 30, 40, 50 and 60 µg/mL by diluting the stock solution.

Evaluation of analgesic and anti-inflammatory action

Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Carageenan induced rat paw edema method

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of KT[24].

Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. KT was administered in dose of 100 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 3 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– KT was administered in dose of 100 mg/kg.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

% inhibition = C-T/ C x 100

C= Paw volume (mL) in vehicle treated group (control)

T= Paw volume (mL) in drug treated group

Tail flick method

The analgesic activity was evaluated using tail flick method[25].

Animals were divided into 3 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)

Group -- II - Standard drug – Ibuprofen

Group – III– KT was administered in dose of 100 mg/kg.

About 5 cm from the distal end, tail of each rat was immersed in warm water maintained at 50°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15\ \text{sec} - \text{reaction time for saline}} X\ 100$$

Results and Discussion

The procured pouches of KT were evaluated for texture, color, taste and odor. The results are shown in table 1. Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug. The results of water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, total ash, water soluble ash, acid insoluble ash are presented in table 2.

Table 1: Organoleptic features of KT

Color	Odor	Taste	Texture
Light Green-Yellowish Green	Odorless	Bitter	Glossy tablets

Table 2: Physicochemical properties of KT

Parameter	Weight of Sample (g)	Weight of ash/extractive (g)	% Value
Total Ash	2	0.162	8.1
Acid insoluble Ash	2	0.047	2.35
Water soluble Ash	2	0.99	4.95
Water soluble Extractives	5	0.068	1.36
Alcohol soluble Extractives	5	0.065	1.3

Total ash value of is an indication of the amount of minerals and earthy materials present in the formulations. KT exhibited 8.1% total ash with 2.35% acid insoluble ash and 4.95% water soluble ash. The water soluble and alcohol soluble extractives were 1.36% and 1.3% respectively suggesting the formulation to be suitable for human use.

The powder of KT was subjected to various chemical tests for preliminary screening of the class of phytoconstituents present in them. The result is presented in table 3.

Table 3: Phytochemical screening of KT

Phytochemical Tested	Observation	Inference
Alkaloid	Cream precipitate formation in Mayer's Test	Present
Glycoside	Greenish color in acetic acid layer in Keller-Killiani Test	Present
Saponin	Frothing Formation	Present
Tannins	Yellow color precipitate in Alkaline Reagent Test	Present
Phenolics	Bluish green color in Ferric chloride Test	Present
Flavonoids	Red color formation in Zinc reduction Test	Present
Proteins and Amino acids	No color formation in Ninhydrin Test	Present
Sterols	Green Color in Burchard Test	Present
Triterpenoids	Grey color in Salkowski Test	Present

As it can be seen from the results all the classes of phytochemicals were present in KT. This is mainly due to the presence of wide variety of plant material (as indicated on the label) in the formulation. The presence of these multiple phytochemicals could easily be linked to the claim of treatment of several disorders by the tablet manufacturer.

TLC Analysis of KT and Curcumin

TLC analysis of KT was done using Curcumin as the marker using ethyleacetate: n-hexane (3:7) as the solvent system. The spots were visusalized using iodine vapors and in UV cabinet. UV Chamber as also used for spotting the other components of KT. Curcumin appeared at Rf value of 0.56 on the TLC plate. Similar spot was obtained in KT indicating the presence of curcumin in the formulation.

Quantitation of Curcumin in KT

Curcumin was eluted using HPLC method employing acetonitrile and 2% v/v acetic acid (40:60) as the mobile phase. Standard curcumin was eluted at retention time 13.117 min using the mobile phase. The HPLC chromatogram of KT exhibited peaks at 1.196, 2.058, 2.169, 3.108, 3.519, 5.591 and 13.121 min owing to the presence of several phytoconstituents that could be eluted out using the mobile phase. The peak at 13.121 min was found due to the presence of curcumin in KT. The quantitation of the curcumin was done from the calibration curve of peak area obtained from standard curcumin and it was found that KT contained 3.75 mg curcumin per 250 mg of KT (1.5 %). This suggests a quite amount of Curcumin in the formulation.

This concentration of curcumin might be responsible for anti-inflammatory and analgesic action in experimental models. Though the other herbs present may contribute towards the action of the formulation. The presence of shatavari also contributes to reasonable amount of anti-inflammatory and analgesic activity in products.

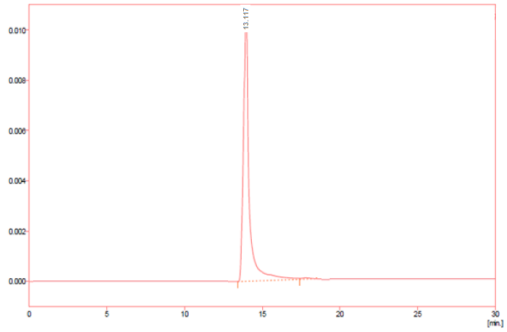

Figure 1:HPLC chromatogram of Curcumin (Retention time 13.117 min, run time 30 min)

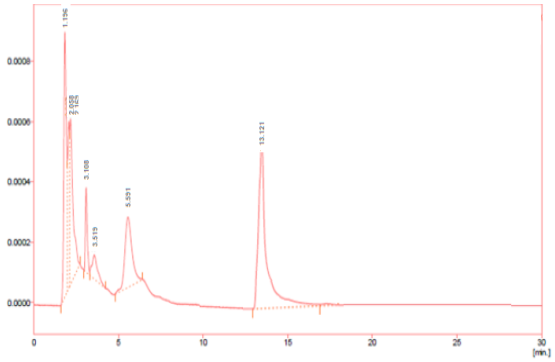

Figure 2: HPLC chromatogram of KT

Evaluation of analgesic and anti-inflammatory action of KT

Table 4 shows the effect of KT and standard drug as compared to the normal saline control at different hours in carageenan-induced rat paw edema model using vernier caliper. Ibuprofen at dose of 10 mg/Kg inhibited 80.80% edema after 4h of administration whereas KT was able to inhibit only 28.27% edema formation.

Table 4: Effect of KT on rat paw edema

Group	Change in Paw thickness (mm) [% inhibition of edema]			
	1h	2h	3h	4h
Normal Saline	1.476 ± 0.025	3.20 ± 0.072	3.82 ± 0.086	4.01 ± 0.047
Ibuprofen	0.48 ± 0.007 [67.47%]	0.93 ± 0.01 [70.94%]	0.96 ± 0.014 [74.87%]	0.77 ± 0.025 [80.80%]
KT	1.27 ± 0.059 [13.97%]	2.47 ± 0.110 [22.81%]	2.56 ± 0.076 [32.98%]	1.95 ± 0.063 [51.17%]

Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. As shown in the table, KT was not able to inhibit edema significantly in the early hours but was able to inhibit edema considerably at 4h. The anti-inflammatory effect of KT was less as compared to Ibuprofen (figure 3).

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. Therefore, it can be inferred that the inhibitory effect of KT on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

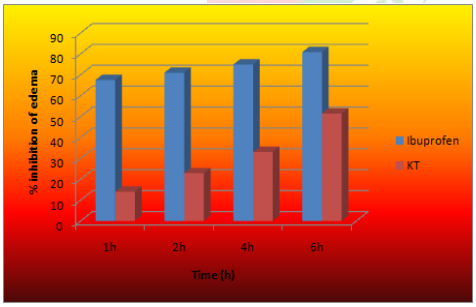

Figure 3: Comparison of anti-inflammatory effect of ibuprofen and KT

The results of analgesic activity of KT by tail flick method are shown in table 5. Rats treated with normal saline (vehicle control) did not exhibit any significant difference in the response time on tail-flick throughout the 60 min duration of observation.

The duration of response time in Ibuprofen and KT was significantly higher as compared to the saline treated animals. The highest reaction time for KT was 6.15 ± 0.242 sec at 30 min post administration while it was 3.46 ± 0.095 sec and 6.22 ± 0.171 sec for vehicle treated group and Ibuprofen respectively at the same time duration.

Table 5: Effect of KT on tail flick response

Group	Response Time in seconds				
	0 min	15 min	30 min	45 min	60 min
Vehicle Control	3.08 ± 0.061	3.22 ± 0.044	3.46 ± 0.0949	3.39 ± 0.058	3.48 ± 0.079
Ibuprofen	4.15 ± 0.128	5.69 ± 0.332	6.22 ± 0.171	6.79 ± 0.142	7.98 ± 0.324
KT	3.53 ± 0.172	4.347 ± 0.358	6.15 ± 0.242	6.00 ± 0.094	5.89 ± 0.095

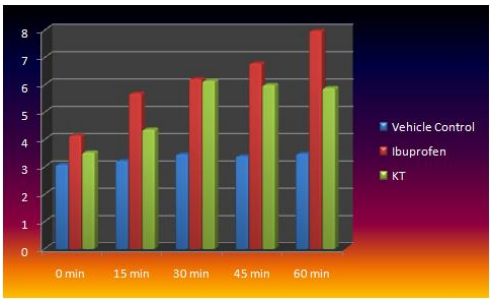

Figure 4: Comparison of analgesic effect of ibuprofen and KT

Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness. The animal model used for screening of analgesic activity in this study is pain-state model using thermal stimuli which includes tail-flick method. The tail-flick method is known to mediate a spinal reflex to a nociceptive stimulus. Ibuprofen was used as the reference drug, which is considered as mild analgesic.

CONCLUSIONS

From the present investigation various standardization parameters such as physicochemical standards like total ash, acid insoluble ash, water & alcohol soluble extractive values, phytochemical analysis, and pharmacological evaluation were carried out, it can be concluded that the formulation Kipen tablets contains good characteristics and it may be harmless for human use.

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