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In Vivo Evaluation of *Sothaghna Karma* (Anti-Inflammatory Activity) of *Bilwa* (Aegle marmelos Corr.) *Moola Twak* and *Patra* - A Comparative Study

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ABSTRACT

One of the characteristics of living tissues is its ability to react to injury, there occurs Inflammation. Inflammation is identified as a symptom according to modern science, whereas in *Ayurveda*, it is correlated to *Sotha,* considered as a separate clinical condition, mentioning its *Lakshana's* (Signs & Symptoms) and *Upadrava* (Complications) in relation with several ailments. Since ancient times, human societies have searched several *Sotha Hara Dravyas*. In the literature of *Ayurveda, Bilwa* (Aegle marmelos Corr.) is mentioned as one of the *Shwayathuhara / Sotha Hara Dravyas* among *Charaka Daseimani Ganas.* The present study is taken up to evaluate the comparative *Sothaghna Karma* (Anti-inflammatory activity) of *Bilwa Moola Twak* and *Patra* of different solvent extracts to standard drug Indomethacin suspension in four different groups of albino rats. On statistical analysis of data Trial drug A when compared with Standard drug, the resultant 't' value showed insignificant difference at P > 0.05. Trial drug B when compared with Standard drug, the resultant 't' value showed insignificant difference at P > 0.05. Both trial drugs and standard drug showed similar results, where as *Bilwa Moola Twak Kashaya* and *Patra Swarasa* has significant action in inhibiting inflammation. Trial drugs in 2nd and 3rd groups showed insignificant difference, having similar anti q-inflammatory action. Based on the observations made and results obtained statistically, the *Bilwa Moola Twak* can be replaced with *Patra* for inhibiting *Sotha*.

Key words: *Sotha, Bilwa Moola & Patra, Anti-inflammatory, In Vivo.*

INTRODUCTION

The entire therapeutical approach of *Ayurveda* is framed upon *Trisutras.*[1] Among them, *Oushadha* is one of the three. Which plays a pivotal role in alleviation of diseases as well as for the maintenance and promotion of good health.[2] The drug (plant, animal or mineral based) is like an instrumental aid to a physician. So, it is also given second utmost importance next to the Physician amongst *Chatushpadas.*[3]

Plants are the integral part of human life. Life depends on plant for daily needs like food, shelter, medicine etc. Also, use of plants for the benefit of human health exists since centuries and even today people are dependent on flora to keep them healthy in some aspects because of easy availability and economic purpose.

Inflammation can be identified as a very common and oftenly affecting ailment in the society. Almost, anything that injures living tissues, there occur the inflammation. Modern science identifies Inflammation just as a symptom found in several ailments.

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Inflammation is defined as the local response of living mammalian tissue to injury, due to any agent. It is often associated with changes that affect the body as a whole, in particular, leucocytosis and fever. Inflammatory reaction is local at first, consisting primarily of changes in blood vessels, the escape of cells and fluids from the blood into the tissues and the consequent changes in the tissues.[4]

Ayurveda signifies Inflammation as a disease in accordance to *Sotha*. *Sotha* can also be seen as a *Lakshana, Upadrava* in several ailments and is one among the *Mahagadas.*[5] *Sotha* is known by many other terms viz., *Sopha, Swayathu, Vranasotha, Vranasopha*. *Sotha* is defined as a thick elevation, *Sama* or *Vishama*, residing between *Twak* and *Mamsa*, due to accumulation of *doshas*, arising in any part of the body.[6]

Several *Sothahara Dravyas* are mentioned in the literature of *Ayurveda*. *Bilwa* (*Aegle marmelos* Corr.) is one among *Swayathuhara Mahakashaya* designed by *Acharya Charaka.*[7] *Acharya Sushruta* and *Vagbhata* included *Bilwa* in *Mishraka Vargeekarana,* the *Bruhat Panchamoolas* and *Dasamoolas* respectively.[8] *Yogaratnakara*, have mentioned *Bilwa Patra* as *Sothahara.*[9] *Bilwa* has been attributed pharmacodynamic properties like *Tiktha - Kashaya Rasas, Laghu-Ruksha Gunas, Ushna Veerya, Katu Vipaka.*[10] As a part of conservation of plants, an attempt is made to replace *Moola* with *Patra*. Considering these points in view, an In Vivo study is taken up to evaluate the *Bilwa Moola Twak* and *Bilwa Patra* for their anti-inflammatory activity keeping the standard drug as Indomethacin.

For experimental study, total 24 albino rats of either sex were randomly selected and divided into four groups of six animals each. Group I as control - treated with normal saline, Group II as standard group - treated with Indomethacin suspension, Group III as Trial drug I - treated with *Bilwa Moola Twak Kwatha*. Group IV as Trial drug II - treated with *Bilwa Patra Swarasa*. Inflammation is induced with carrageenan, paw edema volume was observed and measured. Then, the efficacy of *Sothaghna Karma* (Anti-inflammatory activity) of test drugs with standard drug is noted and statistically analysed.

Materials and Methods

Literary sources

Literary data is collected from the Library of Shri Shivayogeeshwar Rural Ayurvedic Medical College, Hospital and PG Research Centre, Inchal and K.L.E's B.M.K Ayurveda Mahavidyalaya, K.L.E's Pharmacy.

Source of Drug collection

The drug *Bilwa Moola Twak* and *Patra* is collected from the *Dhanvanthari* herbal garden of Shri Shivayogeeshwar Rural Ayurvedic Medical College, Hospital and PG Research Centre, Inchal.

Authentication

The Drug authentication is taken from Mr. Harsha Hegde, Scientist 'B' of Regional Medical Research Centre, Nehru Nagar, Belgaum, the voucher specimen of the same has been deposited in the herbaria of RMRC - ICMR with accession number RMRC - 905.

Preparation of drugs

Bilwa Moola Twak and *Bilwa Patra* were collected, cleaned and dried in shade. The air dried *Moola Twak* and *Patra* were subjected to pulveriser to obtain coarse powder and was stored in air tight container. This was carried out in Teaching Pharmacy of *Rasa Shastra* and *Bhaishajya Kalpana* Department, Shri Shivayogeeshwar Rural Ayurvedic Medical College, Hospital and PG Research Centre, Inchal.

Inclusive Criteria

1. Adult healthy Albino rats of either sex.
2. Albino rats weighing 180-200 gms.
3. Albino rats between 90-120 days old were included.

Exclusive Criteria

1. Unhealthy Albino rats.
2. Weighing within 180 gms and above 200 gms.
3. Albino rats of below 90 days and above 120 days were excluded.

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The animals for experimental study were handled with utmost care to maintain the nutritional status and normal physiological functions.

Albino rats was one of the commonest animals suitable for experimental work because of its size and sensitivity to most of the drugs. It can be bred to obtain pure and uniform strains and was found to be very useful in the studies which withstand long periods of experiment. Hence for the present study, 24 Albino rats were selected and arranged in 4 groups of 6 rats each.

Control Group: 2 ml of 1% Normal saline was fed orally through syringe in a single dose.

Standard Group: Readily available Indomethacin suspension was given to the second group of rats with the help of infant feeding tube and syringe in the dose of 0.9ml/kg body weight.

Test drug 1: The prepared *Bilva Moola Twak Kashaya* was fed orally through syringe tube in the dose of 0.86ml/kg body weight.

Test drug 2: The prepared *Bilva Patra Swarasa* was fed orally through syringe tube in the dose of 0.43ml/kg body weight.

Inflammation induction method

The prepared medicines were administered orally to the respective groups of rats. After half an hour, Inflammation is induced by injecting 0.1 ml of 1% carrageenan into the sub plantar region of left hind paw of all the four groups of rats without disturbing the normal behaviour. Paw volume was recorded immediately just after injecting carrageenan (O hour) and was repeated at 30 mins, 60 mins, 120 mins and 180 mins sequentially. The changes in the paw volume of all groups were compared.[11]

Duration of Treatment

The first seven days was observed for the natural behaviour with suitable housing. Rats were kept starved overnight with water prior to the day of experiment.

On 8th day, administration of test drugs to their respective groups, induction of carrageenan for inflammation and readings of paw edema volume were recorded.

After experiment, the rats were kept for observation for next 5-7 days.

Observation of Anti-inflammatory activity

The rats were handled with good care without altering the normal physiological functions.

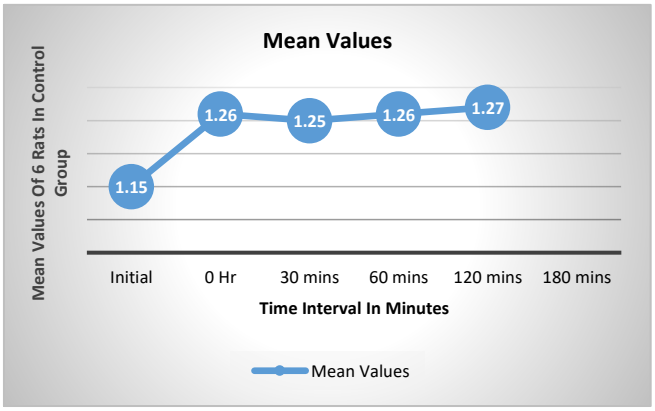
- The hind paw of the rats should be in protruding position.
- The left hind paw (Carrageenan induced) should be measured with Plethysmograph.
- Immersing of the carrageenan induced left hind paw shows rise in mercury level which was measured in ml.
- Reduction of Inflammation was calculated in all groups of rats.

Observations and Results

Table 1: Showing Paw edema volumes (ml) of Control group at different time intervals.

Rats	Initial	0hr	30 Mins	1 Hr	2 Hr	3 Hr
Rat 1	1.15	1.22	1.23	1.23	1.24	1.25
Rat 2	1.13	1.12	1.2	1.19	1.19	1.2
Rat 3	1.17	1.26	1.26	1.27	1.28	1.29
Rat 4	1.12	1.28	1.27	1.28	1.29	1.3
Rat 5	1.15	1.28	1.27	1.29	1.3	1.3
Rat 6	1.16	1.29	1.28	1.29	1.3	1.3
Mean	1.15	1.26	1.25	1.26	1.27	1.27

Graph 1



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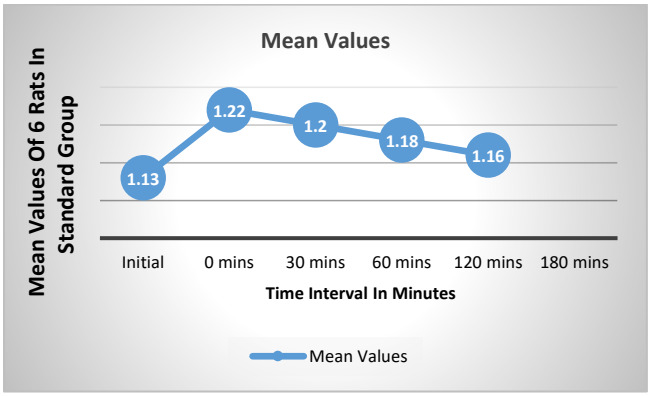
Showing the Mean values of paw edema volume on the X axis and time interval on the Y axis in the Control group (1% Normal Saline).

Table 2: Showing Paw edema volumes (ml) of Standard group at different time intervals.

Rats	Initial	0hr	30 Mins	1 Hr	2 Hr	3 Hr
Rat 1	1.15	1.20	1.16	1.15	1.15	1.13
Rat 2	1.11	1.26	1.23	1.20	1.17	1.14
Rat 3	1.14	1.21	1.20	1.16	1.15	1.13
Rat 4	1.12	1.21	1.18	1.18	1.17	1.15
Rat 5	1.13	1.20	1.20	1.17	1.15	1.15
Rat 6	1.12	1.22	1.21	1.20	1.16	1.14
Mean	1.13	1.22	1.2	1.18	1.16	1.14

Graph 2

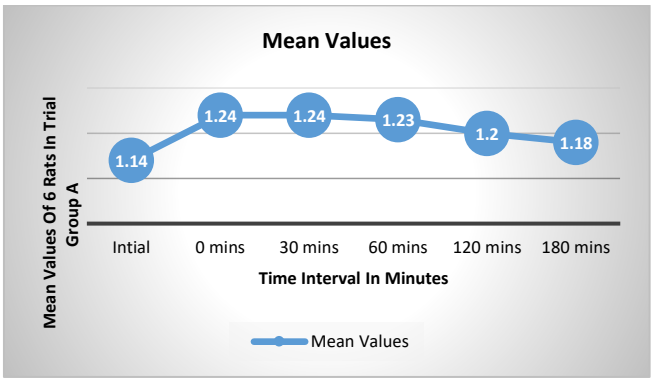


Showing the Mean values of paw edema volume on the X axis and time interval in mins on the Y axis in the Standard group (Indomethacin).

Table 3: Showing Paw edema volumes (ml) of Trial group A at different time intervals.

Rats	Initial	0hr	30 Mins	1 Hr	2 Hr	3 Hr
Rat 1	1.15	1.24	1.23	1.22	1.2	1.19
Rat 2	1.14	1.22	1.22	1.21	1.18	1.16
Rat 3	1.14	1.23	1.25	1.23	1.2	1.18
Rat 4	1.14	1.25	1.24	1.24	1.2	1.19
Rat 5	1.12	1.25	1.25	1.23	1.22	1.17
Rat 6	1.15	1.23	1.22	1.22	1.21	1.2
Mean	1.14	1.24	1.24	1.23	1.20	1.18

Graph 3

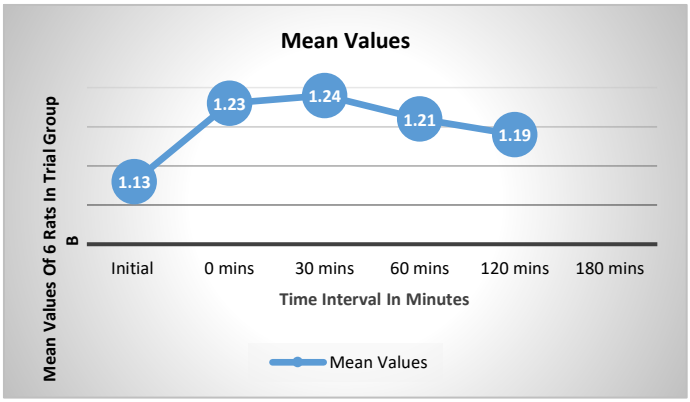


Showing the Mean values of paw edema volume on the X axis and time interval on the Y axis in the Trial group A *(Bilva Moola Twak Kashaya*).

Table 4: Showing Paw edema volumes (ml) of Trial group B at different time intervals.

Rats	Initial	0hr	30 Mins	1 Hr	2 Hr	3 Hr
Rat 1	1.12	1.22	1.24	1.20	1.19	1.17
Rat 2	1.11	1.20	1.22	1.18	1.17	1.16
Rat 3	1.15	1.27	1.26	1.19	1.16	1.15
Rat 4	1.14	1.23	1.26	1.24	1.21	1.18
Rat 5	1.14	1.26	1.25	1.23	1.22	1.2
Rat 6	1.13	1.22	1.23	1.21	1.18	1.17
Mean	1.13	1.23	1.24	1.21	1.19	1.17

Graph 4



Showing the Mean values of paw edema volume on the X axis and time interval on the Y axis in the Trial group B *(Bilva Patra Swarasa*).

After the application of all the four test drugs in all the groups of mice, the volume of reduction in edema is

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noted in ml at specific time interval and statistical analysis is done.

Table 5: Showing the Comparative effect of Test drugs at 30 mins time interval.

Group	S.D.	S.E.	t Value	P < 0.05
Control	0.0084	0.0034	1.47	No
Standard	0.0154	0.0063	3.16	Yes
Trial A	0.0117	0.0048	0.35	No
Trial B	0.0167	0.0068	1.46	No

The above shows, Standard drug has yielded statistically significant results with P < 0.05 as an anti-inflammatory.

Table 6: Showing the Comparative effect of Test drugs at time interval of 60 mins.

Group	S.D.	S.E.	t Value	P < 0.05
Control	0.0117	0.0048	0.35	No
Standard	0.0155	0.0063	6.33	Yes – P < 0.01
Trial A	0.0075	0.0031	3.78	Yes – P < 0.02
Trial B	0.0302	0.0123	2.03	No

From the above table it is found that the Standard and Trial A group has yielded statistically significant results with P < 0.01 and P < 0.02 respectively as an anti-inflammatory.

Table 7: Showing the Comparative effect of Test drugs at time interval of 120 mins.

Group	S.D.	S.E.	t Value	P < 0.05
Control	0.0147	0.0060	1.39	No
Standard	0.0172	0.0070	8.29	Yes P < 0.001
Trial A	0.0105	0.0043	8.17	Yes P < 0.001
Trial B	0.0327	0.0134	3.37	Yes P < 0.02

From the above table it is found that the Standard, Trial A groups has yielded statistically significant results with P < 0.001 and Trial B group with P<0.02 as an anti inflammatory.

Table 8: Showing the Comparative effect of Test drugs at time interval of 180 mins.

Group	S.D.	S.E.	t Value	P < 0.05
Control	0.0152	0.0062	2.42	No
Standard	0.0242	0.0099	7.75	Yes P < 0.001
Trial A	0.0164	0.0067	8.20	Yes P < 0.001
Trial B	0.0293	0.0119	5.16	Yes P < 0.01

From the above table it is found that the Standard, Trial A has yielded statistically significant results with P < 0.001 Trial B groups with P < 0.01 as an anti-inflammatory.

Table 9: Showing comparisons between two specific groups at the time interval of 180 mins.

SN	Group	Difference Between Mean	T Value	P Value
1.	Standard Vs Control	0.0917 ± 0.0117	7.86	< 0.001
2.	Trial A Vs Control	0.0700 ± 0.0091	7.67	< 0.001
3.	Trial B Vs Control	0.0767 ± 0.01345	5.70	< 0.001
4.	Standard Vs Trial A	-0.0217 ± 0.0120	1.81	> 0.05
5.	Standard Vs Trial B	-0.0150 ± 0.0155	0.97	> 0.05
6.	Trial A Vs Trial B	0.0067 ± 0.0137	0.49	> 0.05

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When Standard drug was compared with Control group, the resultant t value was significant at P<0.001. This shows that Standard drug had highly significant anti-inflammatory property when compared to Control.

When Trial drug A (*Bilwa Moola Twak Kashaya*) was compared with Control group, the resultant t value was significant at P<0.001. This shows that Trial drug had highly significant anti-inflammatory property when compared to Control.

When Trial drug B (*Bilwa Patra Swarasa*) was compared with Control group, the resultant t value was significant at P<0.001. This shows that Trial drug B had significant anti-inflammatory property when compared to Control.

When Trial drug A was compared with Standard group, the resultant t value showed insignificant difference at P >0.05. This shows that Standard drug and Trial drug A both have similar anti-inflammatory property.

When Trial drug B was compared with Standard group, the resultant t value showed insignificant difference at P>0.05. This shows that Standard drug and Trial drug B had similar anti-inflammatory property.

When Trial drug A was compared with Trial drug B, the obtained t value showed insignificant difference at P>0.05. This suggests that the anti-inflammatory effect of Trial drugs were similar.

DISCUSSION

In *Ayurvedic* literature, several *Sothahara Dravyas* are mentioned. *Acharya Charaka* quoted *Bilwa* (*Aegle marmelos* Corr.) as one of the drugs in *Swayathuhara Mahakashaya*.

Acharya Sushruta and *Vagbhata* mentioned *Bilwa Moola* as one among *Dashamoolas* and *Bruhat Panchamoolas* respectively. Acharyas like *Yogaratnakara* have mentioned *Bilwa Patra Swarasa* as *Swayathuhara Dravya*. As a part of conservation, in the present study, an attempt is made for the evaluation of anti-inflammatory activity between *Bilwa Moola Twak* and *Patra*.

In Classics, *Sotha* is mentioned as one among the *Asta Mahagadas*. *Sotha* is known by many other terms viz., *Sopha, Swayathu, Vranasotha, Vranasopha*, which may be different in different contexts, but all our *Acharyas* has given much importance to this condition. *Acharya Sushruta* quoted while describing *Nirukti* of *Sopha* as "*Ekadeshootthitha Shopha Ityucchyate*"[12] meaning edema arising in any part of the body is *Sopha*, while mentioning *Vishesha Lakshanas* he uses the word *Swayathu*, which has also been used during description of *Sarvasara Sopha*. *Amarakosha* mentions that the above terms are *Paryayas*. *Sotha* is dealt as a separate disease entity, is also found as a *Lakshana, Upadrava* in several ailments.

Sotha is correlated to Inflammation in contemporary modern science and is identified just as a symptom found in several ailments. The signs of Inflammation are Rubor (redness), Calor (heat), Tumor (swelling), Dolor (pain), Function laesa (loss of function)[13] can be correlated to *Samanya Lakshanas* of *Shotha* like *Anga Vivarnata, Ushma, Utseda, Siraatanutva.*[14]

To understand the disease entity for experiments based on modern science, an attempt has been made by putting correlation between *Sotha* and inflammation according to the respective explanations of the similarities in their symptoms. Till the date, a number of studies have been carried out to screen the various herbs for anti-inflammatory action to put forth the alternative diseases. Herbal remedy, could take the place of synthetic drugs of new era.

The drug *Bilwa* has properties like *Tikta-Kashaya Rasas, Laghu-Ruksha Gunas, Ushna Veerya, Katu Vipaka, Vata-Kapha Naashaka* and actions like *Sothahara, Vedanasthapana, Sangrahi*. The useful parts of *Bilwa* are *Moola, Kanda, Apakwaphala, Pakwaphala, Peshika, Patra*. *Nighantukaras* have mentioned *Rasaadiguna Karmas* on different parts of *Bilwa* separately. *Bilwa Moola Twak* and *Patra* are selected for the present study.

The design of the study on inflammation was made on animal experimentation on Albino rats weighing between 180-200 gms were selected. Four groups of 6 animals each was made based on the following pattern.

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Group I - Control group - 1% normal saline was fed orally to the albino rats at the dosage of 0.2 ml for each animal in the single dose.

Group II - Standard group - Indomethacin suspension was purchased and fed orally to the albino rats at the dosage of 0.9 ml/kg body weight.

Group III - Trial group I - The prepared *Bilwa Moola Twak Kwatha* was fed orally through syringe tube in the dosage of 0.86 ml/kg body weight.

Group IV - Trial group II - The prepared *Bilwa Patra Swarasa* was fed orally through syringe tube in the dosage of 0.43 ml/kg body weight.

Carrageenan 0.1ml was used to induce inflammation in the left hind paw of all the rats. The paw volume of the rats of four groups were measured using plethysmograph at 0 hrs (just after the induction of Carrageenan), 30 mins, 1hr, 2 hr and 3 hr. Observations were done to see the inflammation.

Animal experimentation has its limitation in the evaluation of inflammation, all the symptoms cannot be evaluated as done in clinical study. Only *Utsedha* can be evaluated through the aid of Plethysmograph by which the reduction of inflammation can be calculated.

Inflammation was observed between 15-30 mins after induction of carrageenan, on the analysis of observations, it was found that the albino rats in the control group did not show any improvement by three hours.

Standard group showed improvement from 30 mins whereas Trial A group showed its improvement from first hour and Trial B group showed its inhibition from second hour.

In Inter-group comparisons, Standard group, Trial A group and Trial B group showed highly significant activity against Control group.

When Trial drug A was compared with Standard group, the resultant 't' value showed insignificant difference at P>0.05. This shows that Standard drug and Trial drug A both have similar anti-inflammatory property.

When Trial drug B was compared with Standard group, the resultant 't' value showed insignificant difference at P>0.05. This shows that Standard drug and Trial drug B had similar anti-inflammatory property.

CONCLUSION

Statistically, Standard group, Trial A group (*Bilwa Moola Twak Kashaya*) and Trial B group (*Bilwa Patra Swarasa*) showed significant anti-inflammatory activity. Looking into its anti-inflammatory activity among Trial A group and Trial B group as a part of conservation, *Bilwa Moola Twak* can be replaced with *Patra* for *Sothaghna* property. As per the documented results of experimental study and analysis of Classical literature, it is noted that the reason for the inhibition of inflammation is possible by its pharmacodynamic properties such as *Tikta-Kashaya Rasa, Laghu-Ruksha Gunas* and *Ushna Veerya*.

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