INTERNATIONAL
AYURVEDIC
MEDICAL JOURNAL

IAMJ

Research Article ISSN: 2320 5091 Impact Factor: 4.018

KATUKA RASO SHONITA SANGHATAM BHINNATI" – AN IN VITRO STUDY TO EVALUATE THE THROMBOLYTIC ACTIVITY OF CERTAIN KATU RASA SUBSTANCES

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ABSTRACT

Ayurveda is a science of living beings. *Dravyaguna***,** which is an integral part of a therapeutic effort in *Ayurveda,* uses certain basic principles like *Pancha-Mahabhuta*, *Samanya-Vishesha* & *Rasapanchaka. Rasa* (Taste) of a substance is defined as the total subjective experience arising out of an effective contact between the tongue and the substance. It is used as pharmacological tool in *Ayurveda*. *Acharya Charaka* states that, ***"Katuka Raso Shonita Sanghatm Bhinnatti"*** means substances having *Katu Rasa* would effectuate thrombolysis. *Katu Rasa* substances like *Trikatu* are used by *Ayurvedic* practitioners in the many disease clinical condition. It presents an opportunity to develop a rational therapeutic model and novel drug by taking clue from classical references with the help of In-Vitro study.

Keywords: *Ayurveda, Katu Rasa, In-vitro, Trikatu,* etc

INTRODUCTION

Ayurveda is the part of traditional system of medicine in India. It deals with maintaining the health of a healthy person and helps in the treatment of a patient. It is used in practice since thousands of years of a healthy lifestyle; but due to lack of authentication of procedures on modern scientific parameters it is not being used frequently as conventional system of medicine. *Dravyaguna* is a *Shastra* which helps in gaining knowledge of a particular *Dravya* (drug). It helps in getting knowledge about the properties, uses, advantages and disadvantages of a particular drug or their formulations. *Rasa* itself is a specific quality of a *Dravya* which comes in knowledge after contacting with *Rasendirya* (tongue). There are six types of *Rasas* are mentioned in *Ayurvedic* texts: *Madhura*, *Amla*, *Lavana*, *Katu*, *Tikta* and *Kashaya*[1]. *Katu Rasa* is a composition of *Vayu* & *Agni Mahabhutas*[2] which possesses qualities *Laghu, Ruksha, Usna* & *Tikshna*. It stimulates tongue and increases secretions from mouth and nose when comes in contact with tongue. It also produces burning sensation, helps in digestion, and cleans the oral

cavity. Blood coagulation which causes many serious health problems like myocardial infarction [MI], deep vein thrombosis, renal vein thrombosis etc., which already have a well mentioned treatment in conventional system of medicine; but in traditional system of medicine there is a quotation given by *Ayurvedic Acharyas* which are still not used in our regular practices due to lack of there scientific authentication. *Acharya Charaka* mentioned ***"Katu Rasa Shonita Sanghata Bhinnati"***[3] which states that *Katu Rasa* is having ability to breakdown the blood clot. This *Sutra* gives us a hope to make a management of blood coagulation disorders without any complication. Blood coagulation disorders are increasing day by day. There is vast treatment for these disorders in conventional system of medicine, but they having their own side effects. So it's our scientific approach to assess **"***Katuka Raso Shonita Sanghatam Bhinnati*" – an invitro study to evaluate the thrombolytic activity of certain *Katu Rasa* substances**".**

OBJECTIVES

- Classical references of "*Katu Rasa Shonita Sanghata Bhinnati"* in different text.
- Pharmacognostical analysis of selected drugs.
- To evaluate the thrombolytic activity of selected drugs in In-vitro.

Material and Methods-

Blood specimen- With all aseptic conditions, 5 ml of venous blood will be drawn from healthy volunteers without a history of oral contraceptives or anticoagulant therapy. 500 µl bloods will be transferred into 10 pre-weighed sterile micro centrifuge tubes. These tubes will be incubated at 37°C for 45 minutes. After clot formation, serum will be completely removed (aspirated out without disturbing the clot formed) and each tube having clot will be again weighed to determine the clot weight [W1] (clot weight = weight of clot containing tube – weight of tube alone).

Positive control- Add 5 ml sterile distilled water to Streptokinase vial (15,00,000 IU) and mix properly. This suspension will be used as a stock from which 100µl (i.e. 30,000 IU) will be used for each micro centrifuge tube containing blood clot. Stock solution should be used on the same day.

Negative control- 100µl distilled water will be used for each micro centrifuge tube containing blood clot.

Drug Groups-Add drugs as per dose decided to each micro centrifuge tube.

All the tubes after adding drug samples, positive and negative control will be incubated at 37°C for 90 minutes and will be observed for clot lysis. After incubation, fluid released will be removed and tubes will again be weighed to get the clot weight after clot lysis. [W2].

Assessment criteria-Difference of clot weight before and after clot lysis (W1 – W2) will be expressed as percentage of clot lysis. Experiment needs to be repeated at least 3 times with blood samples of the volunteer.

X1 = wt. of empty tube
X2 = wt. of tube with clot
X4 = wt. of tube after incubation with drug
WXG = wt. difference

[W1] X3 = X2 - X1
[W2] X5 = X4 - X1
WXG = [W2] X5 – [W1]X3

Statistical analysis:[4]

ANOVA test is used for statistical analysis of study. It is also called the 'F' test which is developed to test the homogeneity of more than two sample means. As the variance between the groups is analyzed, the procedure is also known as Analysis of Variance (ANOVA).

In F-test the total variability is divided into two

- Variability due to assignable cause (treatment effect) or difference between two groups.
- Variability due to chance cause (unknown effect) or difference within the group or experimental error or simply 'Error'. It is expressed as a ratio of the difference between groups and hence known as 'F-ratio'.

$$F = \frac{\text{Variability due to assignable causes per d.f}}{\text{Variability due to chance cause per d.f.}}$$

$$F = \frac{\text{Mean variability between groups}}{\text{Mean variability within groups (Error)}}$$

$$F = \frac{\text{Mean sum of squares between the groups}}{\text{Mean sum of squares within the groups}}$$

The research conducted in 14 volunteers. It's an Interventional type, Non-Randomized, Active Controlled Trial study designee which including 14 groups. The volunteers selected for trial were the students of BKAMCH, Daudhar, Moga, and Punjab.

Inclusion criteria

20-25years age group of both gender healthy volunteers has taken which do not have any history of anticoagulant therapy and oral contraceptives.

Exclusion criteria

Unhealthy volunteers which having any history of anticoagulant therapy and oral contraceptives.

Subjective parameters

Sign & symptoms of a healthy person according to *Ayurveda* for healthy volunteers. Persons have proportionate musculature and compactness of the body. They can stand hunger, thirst, the heat of the sun, cold and physical exercises. They can digest and assimilate properly[5]. In modern the criteria for health assessment like CBC; ESR; BT-CT; Urine Complete.

Objective parameters- The experiment will be carried out in following fourteen groups.

Test Group	Test Group
1. **Control Group -** Water.	8.**Test Group VI -** *Zingiber officinale* 25 mg.
2. **Standard Group -** 0.1 ml (Inj. Streptokinase)	9. **Test Group VII -** *Piper nigrum* 100 mg.
3. **Test Group I -** *Piper longum* 100 mg.	10. **Test Group VIII -** *Piper nigrum* 50 mg.
4. **Test Group II -** *Piper longum* 50 mg.	11. **Test Group IX -** *Piper nigrum* 25 mg.
5. **Test Group III -** *Piper longum* 25 mg.	12. **Test Group X –** *Trikatu* 100 mg.
6. **Test Group IV -** *Zingiber officinale* 100 mg.	13. **Test Group XI -** *Trikatu* 50 mg.
7. **Test Group V -** *Zingiber officinale* 50 mg.	14. **Test Group XII -** *Trikatu* 25 mg.

OBSEVATIONS

Table 1: Health wise distribution

S.no.	Health status	No. of volunteers
1.	*Swastha Purusha*	14
2.	*Aswastha Purusha*	6

(Graph no.01)

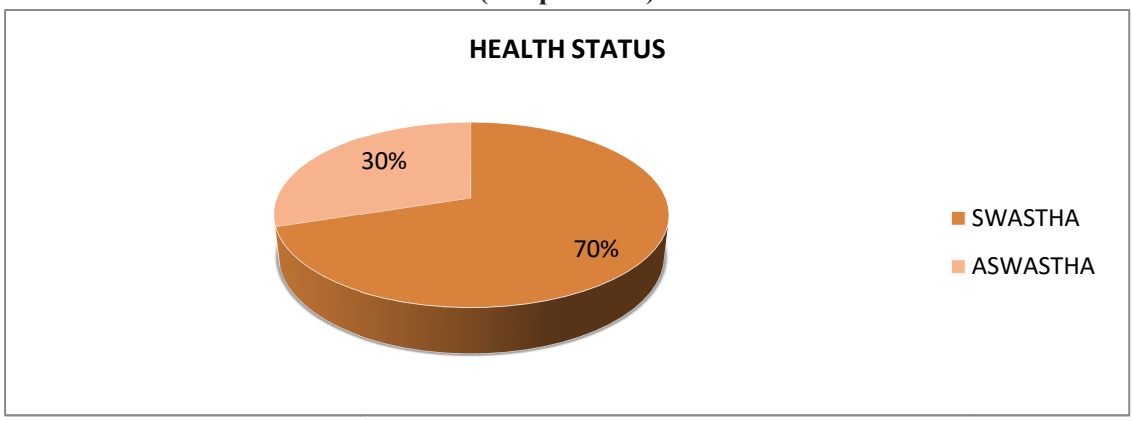

Table 2: Distribution on the basis of subjective parameter of 20 volunteers

S.no.	***Swasth Purusha Lakshana***	**No. of volunteers**
1.	*Shritsaha*	18
2.	*Pipasasaha*	20
3.	*Aatapsaha*	20
4.	*Seetsaha*	**14**
5.	*Vyayamsaha*	**14**
6.	*Sampkta*	20
7.	*Samjra*	20
8.	*Sam-mansa*	**14**

(Graph no.02)

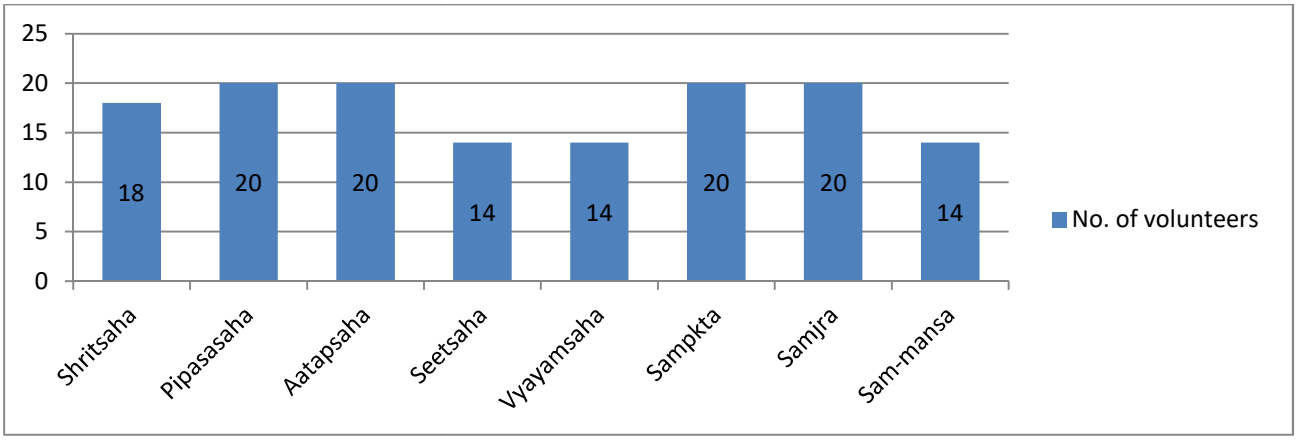

Table 3: Distribution on the basis of general, haematological laboratory investigations

S.N.	**General, haematological laboratory investigation**	**No. of volunteers**
1.	CBC	18
2.	ESR	**14**
3.	BT-CT	19
4.	Urine Complete	20

(Graph 03)

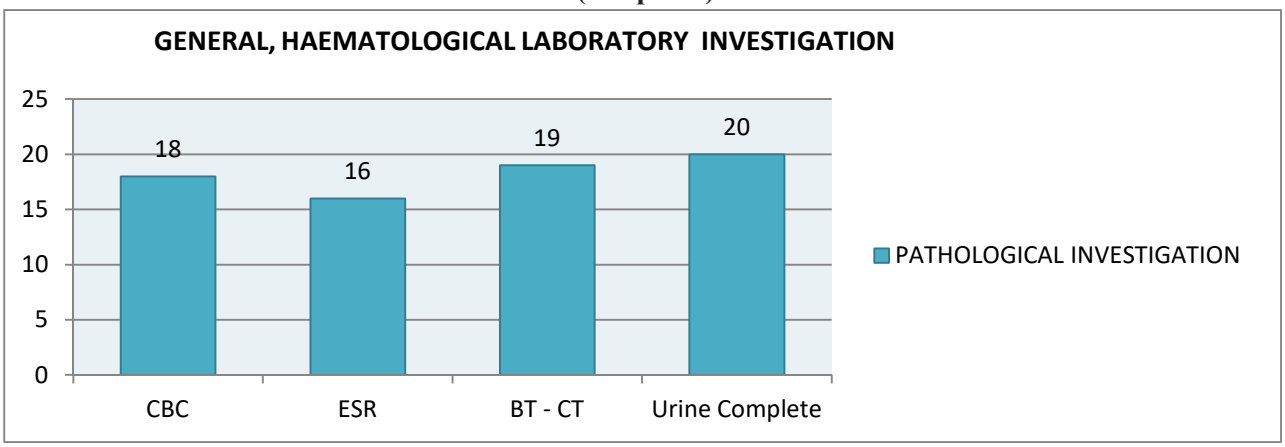

Table 4: Statistical Analysis

	W CG	W SG	W TG1	W TG2	W TG3	W TG4	W TG5	W TG6	W TG7	W TG8	W TG9	W TG10	W TG11	W TG12
R^2	1.000	1.000	0.733	1.000	0.481	0.481	1.000	1.000	0.711	1.000	1.000	0.953	1.000	1.000
F			5.917		2.000	2.000			5.302			43.905		
Pr > F			< 0.0001		0.061	0.061			0.000			**< 0.0001**		

- ANOVA Test is done at the level of 95 %.
- According to statistical analysis WTG10 is < 0.0001 at level of 95% which shows that our research hypothesis is accepted.

CTRI Registration-
CTRI/2018/03/012778 [Registered on: 23/03/2018] - Trial Registered Prospectively

DISCUSSION

Rasa is a sensation of taste observed by tongue. It is of six types; mentioned about in all the *Ayurvedic* classical texts i.e. *Madhura, Amla, Lavana, Katu, Tikta* and *Kashaya*. *Acharya Charaka* mentioned a quote "***Katu Rasa Shonita Sanghatam Bhinnati***" on the basis of his observations and theories; in modern scenario we are not considering these all concepts in research without any proper research. To prove "***Katu Rasa Shonita Sanghatam Bhinnati***" we take *Katu Rasa* enriches drugs and preparations. We choose *Trikatu* including *Pippali, Marica,* and *Sunthi.*

Katu Rasa is having *Guna's* (properties) like *Laghu, Ruksha, Usna* and *Tikshna*. These four *Guna's* are mentioned in *Guruadi Guna* in *Samhita's*[6]. From all of these *Laghu Guna* is having *Pradhanta* (domination) of *Akasha, Vayu* and *Agni Mahabhuta's*. It gives lightness to the body and helps in breakdown of macro particles into micro particles[7]. If we are talking about the *Ruksha Guna* of *Katu Rasa* which is itself a specific property of *Vata Dosha*. *Vata* is responsible for *Vibheda* (differentiation) due to its *Prabhav* (impact factor). The *Usna* property of *Katu Rasa* helps in increasing the temperature. It helps in liquefaction of blood, due to having dominancy of *Vayu* and *Agni Mahabhuta* in it. *Tikshna Guna* of *Katu Rasa* produces burning sensation and causes secretion due to dominancy of *Agni Mahabhuta*. Whole four properties of *Katu Rasa* show the *Pradhanta* of *Vayu* & *Agni*. We can say that due to *Vayu, Katu Rasa* is having an ability to move in channels very easily and quickly. *Vayu* can enhance the "***Chal Guna***" and *Agni* is responsible for

Dahakarak or liquefaction of "***Shonita Sanghata"***. *Laghu, Ruksha, Usna, and Tikshna;* they finally shows as synergism effect of "***Shonita Sanghatam Bhinnati***" that also known as thrombolysis of blood.

Pippali is mentioned in almost all the *Ayurvedic* classical texts. *Acharya Charaka* mentioned *Pippali Dravya* in *Charaka Samhita Chikitshasathana*[8] and *Kalpsathana*[9]. In *Charaka Samhita Pippali* is also described as *Pippali Vardhmaan Rasayana*[10]. The varieties of *Pippali* are described by both *Sushruta* and *Vaghbhatta*. *Pippali* is mentioned in about all the *Nighantu's* like *Raj Nighantu, Dhanwantri Nighantu, and Bhavprakasha Nighantu* etc in different *Vargas*.

The description of ***Marica*** is found in all *Ayurvedic* Texts *Samhita's* as well as in *Nighantu's* also. *Marica* is mentioned in *Vatajanya Chardi Rog Chiktisha* in *Charaka Samhita Chikitsasthana*[11]. In *Sushruta Samhita Marica* is explained in *Sutrasthan*[12]. In *Astanga Sangraha*, *Marica* is explained in *Sutrasthana*[13]. About all in all the *Nighantus* the description of *Marica* are mentioned.

Sunthi is described in almost the *Ayurvedic* texts due to its medicinal values. *Acharya Charaka* mentioned *Sunthi* in *Chikitsasthana*[14]. In *Sushruta Samhita, Sunthi* is described in *Sutrasthana*[15]. *Sunthi* is mentioned in almost all the *Nighantu's* in different *Vargas*.

Trikatu is the combination of three *Dravyas* having *Katu Rasa;* the three *Dravyas* are *Pippali, Marica* and *Sunthi* respectively. It is mentioned in almost all the *Ayurvedic* texts. ***Acharya Charaka*** mentioned it in *Chikitsasthana*[16]. In ***Sushruta Samhita*** it is mentioned as *Pippali, Marica* and *Sringvera (Sunthi)* together form *Trikatukam*[17]. According to ***Acharya Sharangdhar***[18] and ***Madanpal Nighantu***[19] *Pippali, Marica* and *Sonth* collectively known as *Tryusana*. In ***Kaiyadev Nighantu***[20] the description of *Trikatu* is mentioned in *Aushadh Varga*. *Acharya Priyavata Sharma Ji* mentioned *Trikatu* in *Pippalyadi Varga* of ***Priya Nighantu***[21]. *Trikatu* is mentioned in *Haritkyadi Varga* of ***Bhavprakasha Nighantu***[22]. *Trikatu* is mentioned in *Misraka Varga* of ***Dhanwantri Nighantu***[23]. *Trikatu* is mentioned in *Miskrakadhya Varga* of ***Sodhal Nighant***[24] *&* ***Raj Nighantu***[25]**.**

In **In-Vitro** study we take history of 20 volunteers. From which 14 volunteers are selected for trial. Because on the basis of subjective criteria (**"*Swastha Purusha Lakshana"***[26] as mentioned in *Charaka Samhita* & General & Haematological Investigations). According to subjective criteria of *Swastha Purusha*; **(Table no.2)** all eight parameters (*Lakshana*) found in 14 volunteers, and remaining six volunteers are not found positive for these parameters **[Table no. (1)]**. In haematological investigation out of twenty volunteers we found fourteen volunteers **(Table no.3)** between normal ranges. Both *Shastrokt Swastha* criteria and lab investigation shows that our fourteen volunteers are not suffering from any diseased condition especially thrombolytic disorders **(Table no.2)**. That means our all selected volunteers are *Swastha*.

According **(Table no.4);** we found that *Trikatu* (**WTG10** which contains ***Trikatu* 100 mg**) is having more efficient of thrombolysis activity then *Pippali* (**WTG1** which contains ***Pippali* 100 mg**) and then *Marica* (**WTG7** which contains ***Marica*100 mg**) and then gradually *Pippali* (**WTG3** which contains ***Pippali* 25 mg**) & *Sunthi* (**WTG4** which contains ***Sunthi* 100 mg**) are same. *Trikatu* have more *Usna, Tikshna* properties comparatively to others thus it shows more thrombolysis effect on In-vitro research, whereas gradually *Marica, Pippali* and *Sunthi* have comparatively less *Usna, Tikshna* properties so that they shows comparatively less thrombolysis activity in our research work.

Finally we can conclude that in our research work, *Katu Rasa Pradhana Dravya* and formulations have thrombolysis activity in In-vitro study. So it's clear that ***"Katu Rasa Shonita Sanghata Bhinnati"*** which is mention in our *Charka Samhita* is significantly proven on modern research parameters.

CONCLUSION

Ayurveda is an absolute holistic tradition that reaches far beyond the limits of physical health, healing and the prevention of diseases. It is a system that is authentic and reliable. Since *Ayurveda* is preventive, as well as holistic and curative, it can lead the world closer to a disease-free society.

Dravyaguna is also a part of *Ayurveda* which contains a full detail about the drugs and there uses in different conditions. In *Ayurvedic* texts it is clearly mentioned that ***Katu Rasa*** have ability to treat the thrombosis but due to lack of its authentication no one tried to use this for these conditions. In this in-vitro study we proved that the quotation given by our *Ayurvedic Acharaya's* is correct that *Katu Rasa* is having ability to break the blood clots. In-vitro study is like the first level to prove the authentication of the quotation ***"Katu Rasa Shonita Sanghata Bhinnati"*** there is a scope for further research on this topic. The In-vivo (both animal trial and clinical trial) study for this topic can be the second stage to prove its authentication in a proper scientific manner, which can helps us to use new drug in management of thrombogenic life threatening diseases.

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Source of Support: Nil
Conflict Of Interest: None Declared

How to cite this URL: Ritu Rani & Sandeep Singh Tiwari: Katuka Raso Shonita Sanghatam Bhinnati" – An In Vitro Study To Evaluate The Thrombolytic Activity Of Certain Katu Rasa Substances. International Ayurvedic Medical Journal {online} 2018 {cited November, 2018} Available from: http://www.iamj.in/posts/images/upload/1413_1420.pdf