International Journal of Health Sciences and Research
Vol.12; Issue: 8; August 2022
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

To Evaluate Antimycobacterial Activity of *Rasamanikya* by Using Advance Bact/ALERT 3-D Automated Liquid Culture WSR to Tuberculosis

Dr. Yogesh Madgulwar[1], Dr. Vinod Ramteke[2], Dr. Raman Belge[3]

[1]P.G. Scholar, Dept. of Rasashastra & Bhaishajya Kalpana, Shri Ayurved Mahavidyalaya, Nagpur
[2]P.G. Guide, Associate Professor, Dept. of Rasashastra & Bhaishajya Kalpana, Shri Ayurved Mahavidyalaya, Nagpur
[3]Professor & H.O.D. Dept. of Rasashastra & Bhaishajya Kalpana, Shri Ayurved Mahavidyalaya, Nagpur

Corresponding Author: Dr. Yogesh Madgulwar

DOI: https://doi.org/10.52403/ijhsr.20220830

ABSTRACT

Tuberculosis is respiratory disease. Tuberculosis generally affects the lungs, but it can also affect other parts of the body. Latent tuberculosis in which case no symptoms seen. Around 10% of latent infections progress to active disease which, if left untreated, kill about half of those affected. Typical symptoms of active TB are chronic cough with blood containing mucus, fever, night sweats and weight loss. Tuberculosis spread from one person to another person when people who have active TB in their lungs cough, spit, speak or sneeze. People with latent TB don't spread the disease. There is no direct reference of tuberculosis in ayurveda but we could consider it under Rajaykshma by its signs and symptoms. Rajaykshma is group of disease which includes vitiation of tridosha and Sapta dhatu, due to indulging in Sahasa, Vega Sandarana, Kshaya, Vishmashana, there is manifestation of Ekadasha Rupa. Rasamanikya is a herbo-mineral formulation indicated for Rajaykshma. Rasamanikya is a Kupipakwa Rasayan. It was prepared according to ref. of Brihatrasarajasundar mentioned in Rajaykshmarogadhikar. Hence it was chosen to evaluate antimycobacterial activity by using Bact alert 3 D automated liquid culture.

Aim: To evaluate antimycobacterial activity of Rasamanikya by using advance Bact/ALERT 3-D Automated liquid culture WSR to Tuberculosis.

Objective:

To prepare Rasamanikya

To have analytical test of Rasamanikya and to evaluate antimycobacterial activity by using Bact alert 3-D automated liquid culture.

Material And Methods: Rasamanikya was prepared according to ref mentioned in Brihatrasarajasundar. Analytical tests of Rasamanikya were performed according to methods described in ayurvedic pharmacopoeia of india and antimycobacterial activity of Rasamanikya was carried out with the help of Bact alert 3-D automated liquid culture.

Results: Rasamanikya extract at MIC 1g/ml was able to achieve significant ($P < 0.001$ vs MTB culture & Other concentration of herb) antimycobacterial activity which was comparable to MIC of TB drugs. Another concentration of Rasamanikya although showed antimycobacterial activity based on delayed growth time compared to MTB culture however, they are not significant compared to TB drugs.

Keywords: Tuberculosis, Antimycobacterial activity, Bact alert 3-D automated liquid culture system, Rajayakshma, Rasamanikya.

INTRODUCTION

Tuberculosis (TB) is a contagious illness generally caused by mycobacterium tuberculosis bacteria (MTB). Tuberculosis generally affects the lungs but it can also affect other corridor of the body. Utmost infections show no symptoms, in which case it's known as tuberculosis. Around 10 %of infections progress to active ailment which, if left undressed, kill about half of those affected [1]. Typical symptoms of active TB are habitual cough with fever, night sweats and weight loss. It was historically referred to as consumption due to the weight loss associated with the illness. Infection of other organs can be getting a wide range of symptoms [2]. Tuberculosis is spread from one person to other person through air when people who have active TB in their lungs cough, spit, speak, sneeze. Active infection occurs more frequently in people with HIV/ AIDS and in those who smoke. Diagnosis of active TB is Based on chest X- rays, as well as microscopic examination and culture of TB of body fluids. Diagnosis of Latent TB relies on the tuberculin skin test (TST) or blood tests. In ayurveda Rajayakshma can be correlated with tuberculosis of modern science. Rajayakshma is a Madhyama rogamarga Vyadhi manifested by Shosha[4] as mentioned in table 1. Due to indulging in Sahasa,Vega Sandharana, Kshaya, Vishamashana, there's the illustration of Ekadasha Rupa where it affects the Tridosha and Sapta Dhatu[5]. Ayurveda classics gives significance to Nidana because it's a Sarva Tantra Siddanta that effect will always be going to imitate the cause i.e., Karya Karana Bhava Before understanding Chikitsa the knowledge of nidana and the samprati is necessary [6].

Rogamarga	Structures	Diseases
Bahya	Rakta, Mamsa, Meda, Majja, Shukra, Twak	Ganda, Pidaka, Alaji, Apachi, Visarpa, Arsha,Gulma, Charmakeela, Shvayathu, Vidradhi
Madhyama	Marma, Asthi, Sandhi, Snayu, Kandara	Pakshavadha, Paksha Graha, Shosha, Ardita Apatanaka, Rajayakshma
Abhyantara	All Kostangas	Jvara, Murcha, Alasaka, Kasa,Gulma, Hikka, Arsha, Visuchika, Anaha, Pleeha, Visarpa, Shvayathu, Vidradhi, Athisara

Table 1- Representing Rogamarga and structures

Chikitsa is nothing but the Nidana Parivarjana and Samprapti Vighatana. Rasamanikya is mentioned in Brihatrasarajasundar in Rajaykshma rogadhikar [7]. It contains Shuddha Parad[8] (Mercury), Shuddha gandhak[9] (Sulphur), Shuddha Manshilla[10], (Realgar) Shuddha naga [12] (Lead).This experimental study was done with the end to estimate antimycobacterial exertion of Rasamanikya using 3-D automated liquid culture system.

MATERIAL AND METHODS

Present study was conducted under 3 headings:

1. Pharmaceutical study
2. Analytical study
3. Experimental study

1. Pharmaceutical study:

Rasamanikya was prepared by the traditional method given by the Acharya "Brihatrasarajsundar" mentioned in Rajyakshmarogadhikar. This section comprises of

1. 1.Shodhana of Parad, Gandhak, Manshilla, Naga
2. 2.Preparation of kajjali of Rasamanikya
3. 3.Preparation of Rasamanikya [kupipakwa Method] [13]

Shodhana (Purification)

Shodhana of Parad (Mercury), Gandhak (Sulphur), Manshila (Realgar), Naga (lead) was carried according to classical reference given the book.

Preparation of Rasamanikya: [Kupipakwa method]

Rasamanikya was prepared according to ref of Brihatrasarajsundar. Rasamanikya kajjali was prepared. Then, Kajjali was filled in Kupi. Kupi was given heat in Kramagni pattern i.e, Mrudu, Madhya, Teevragni.
After all, Kupipakwa siddha lakshan heat stopped. Kupi was left for Swangsheeta. Then Kupi bhedan was done. Rasamanikya product was deposited at neck portion of kupi.

2. Analytical study
All the parameters required for testing of tablets like pH, L.O.D, Total ash-value,Water soluble Extract, XRD was carried out accordingly and findings were noted.
Analytical tests suggest Total ash 5.54%, Acid Insoluble ash 0.16%, Water soluble ash 2.75%, loss on drying at 105℃ 0.11%, pH of 10 & aqueous solution 6.32.XRD study shows HgS as a chemical constituent.

Materials and Methods

Preparation of stock solutions of the selected compounds:
Stock solutions of the Ayurvedic mineral drug was prepared by dissolving 62.5 mg of Rasamanikya in 1ml of autoclaved distilled water to make final concentration of 62.5mg/ml.
Preparation of TB drugs stocks for Minimum Inhibitory concentration (MIC) studies. Standard TB drugs isoniazid and rifampicin was purchased from CIIMS pharmacy department. The drugs were crushed using sterile pestle mortar. The drug powder were dissolved in respective solvent (ethanol~rifampicin, methanol~ isoniazid) at concentration of 1.25 mg /25µl each drug to make final concentration 50µg/µl.

Standardization and optimization of the Growth curve for MIC study (17,18,20,21)
Clinical isolate of Mycobacterium tuberculosis (MTB), H 37 Rv strain was procured from CIIMS microbiology laboratory and was confirmed through the Acid-fast staining. For the studies, the selected MTB culture was first grown till mid log phase by inoculating 0.5-1ml (~10 7 CFU) suspension of culture in Middlebrook liquid medium supplemented with oleic acid ovalbumin dextrose calatase (OADC) enrichment (Biomeriux, France) followed by incubation in automated liquid culture system BACT/alert 3D (Biomeriux, France). The time taken for positive growth was used for standard curve generation.

To determine the minimum inhibitory concentration (MIC) of standard anti-TB drugs cocktail using automated liquid culture system (18,20,21,25)
MIC of standard anti-TB drugs was determined by inoculating MTB culture along different dilutions of isoniazid (50µg/µl-100 µg/µl) and rifampicin (25µg/µl-50 µg /µl) in middlebrook OADC media in BacT/alert system at 37 o C. Standard culture without any drugs will be taken as the positive control. MICs were defined as the lowest drug concentration of the drugs that inhibits the growth of more than 99.0% of a bacterial proportion of the tested MTB strains in liquid culture.
To evaluate anti-mycobacterial activity of Rasamanikya extract using advance BacT/ALERT 3D automated culture system (17,18,20,21,25)
MIC of ayurvedic mineral drug Rasamanikya along with MTB culture was determined by inoculating MTB culture along with different dilutions of extracts of in Rasamanikya BACT/alert system at 37 ℃. For preparation of different concentration of Rasamanikya, different serial dilutions of stock concentration was made (62.5mg, 46.87mg, 31.25mg,15.62 mg) in appropriate volumes of sterile saline. Standard culture without any drugs was taken as positive control. MIC concentration of anti TB drugs that were earlier evaluated were also taken in experimental sets for comparative studies with herbal extract.
Group 1: MTB culture only (positive control)
Group 2: MTB culture+ TB drugs

Group 3: MTB culture + Ayurvedic herbo mineral drug #
[Note: # different dilution (62.5mg, 46.87mg, 31.25mg, 15.62 mg) of extract of Rasamanikya was evaluated]

To study synergistic anti-Mycobacterial activity of Rasamanikya along with TB drugs on MTB using BACT alert
(19,25,26)
To study synergistic activity, both drugs and extract of Rasamanikya were inoculated together in MIC values and concentration below their MIC values. The concentration below
MIC values was used to study whether lower concentration of drugs in herbal formulation
can inhibit MTB growth.
Following sets will be used

Group 1: MTB culture only (positive control)
Group 2: MTB + Rasamanikya # + TB drugs (concentration below MIC)
[Note: # different dilution (62.5mg, 46.87mg, 31.25mg, 15.62 mg) of extract of Rasamanikya was evaluated]

RESULTS

Standardization of growth curve of MTB H37Rv: Standard growth curve of MTB H 37 Rv was grown by inoculating 10 7 CFU of MTB strain in automated BacT/ALERT 3D machine. The overall mean time taken by the culture to show positivity was considered as standard growth time. This standard growth time was used as positive control for remaining sets to study antimycobacterial activities of Rasamanikya & rifampicin, isoniazid; TB drugs.

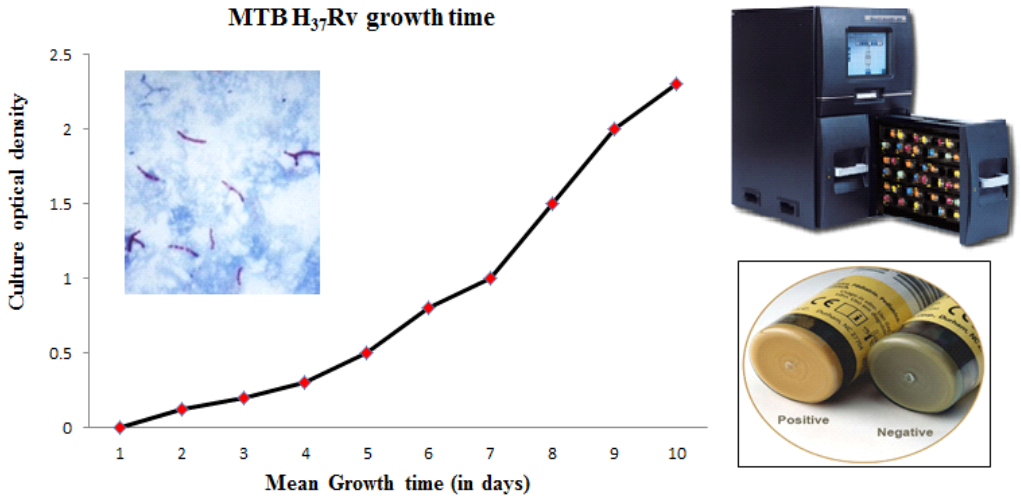

Figure1: Mean growth time of clinical MTB H 37 Rv clinical isolate after its inoculation in BacT/ALERT 3D machine.

The positive growth was further confirmed by Acid fast staining confirming MTB. Standard growth cure of MTB after its inoculation in BacT/ALERT 3D machine is shown in Figure 1. Based on results, standard growth time for mid log phase culture was recorded to be around 10 days. The positive culture was further confirmed by Acid fast staining and observation under oil immersion microscopy. MTB was observed as Pink slender rod-shaped bacilli in microscopy.

Estimation of minimum inhibitory concentration (MIC) of standard anti-TB drugs cocktail using automated liquid culture system.
Drug susceptibility test against standard drug was conducted by inoculating stock concentration of Rifampicin and isoniazid with mid log phase culture in automated BacT/ALERT 3D machine.
Mean delay in growth time compared to positive control (culture without drugs) was taken as MIC for MTB drugs. Based on the results, the MIC for Rifampicin was found

to be 50µg/µl, while that for isoniazid was found to be 100µg/µl. which was significantly ($p < 0.001$) associated for inhibition of bacterial growth compared to MTB culture without any drug.

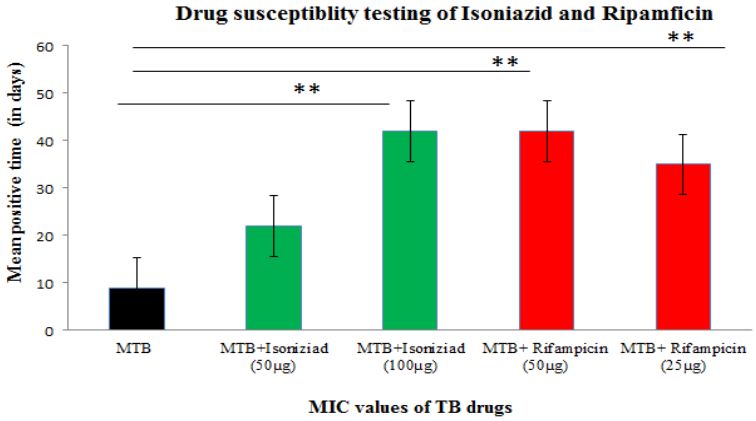

Figure 2: Drug susceptibility testing and MIC evaluation of isoniazid and rifampicin against MTB cultur BacT/ALERT 3D machine.

In figure 2, each bar represents mean growth time (of triplicates) of MTB after inoculation different drug concentrations. The MIC values represents mean delay growth time compared to MTB culture without any drugs. Standard 42-day protocol was considered as complete inhibition of MTB growth ** represents statistically significant values ($P < 0.001$) compared to MTB culture.

To evaluate anti-mycobacterial activity of Rasamanikya extract using advance BacT/ALERT 3D automated culture system.

To study anti-mycobacterial activity of Rasamanikya, different dilutions of Rasamanikya extract was inoculated with mid log phase MTB culture in automated BacT/ALERT 3D machine. Standard drugs at MIC values were also used as reference standard to compare antimycobacterial effects of Rasamanikya. Each experimental set was carried out in triplicates. Based on the results, it was found that Rasamanikya extract at MIC 1g/ml was able to achieve significant ($P < 0.001$ vs MTB culture & other concentration of herb) antimycobacterial activity which was comparable to MIC of TB drugs. Another concentration of Rasamanikya although showed antimycobacterial activity based on delayed growth time compared to MTB culture, however, they are not significant compared to TB drugs.

Overall, MIC values of TB drugs as observed from studies are mentioned in Table 1.

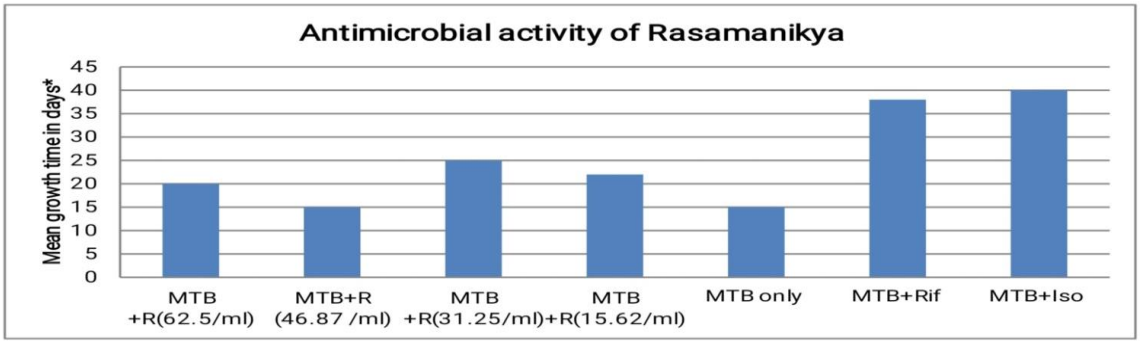

Figure 3 - Antimycobacterial activity of Rasamanikya

Figure 3: Antimycobacterial activity of different concentrations of Rasamanikya (A) against MTB culture in BacT/ALERT 3D machine. Standard drugs Rifampicin (RIF) and Isoniazid (INH) were taken as reference standard, Each bar represent mean growth time (of triplicates) of MTB after inoculation. The MIC values represents mean delay growth time compared to MTB culture without any drugs.

To study synergistic anti-Mycobacterial activity of Rasamanikya along with TB drugs on MTB using BACT alert

To study synergistic anti-Mycobacterial activity of Rasamanikya with TB drugs, different dilutions of herb at MIC and below their MIC were inoculated with MTB culture in BacT/ALERT 3D machine. The details of experimental set and mean delay in growth time with syngergistic combination of TB drugs with Rasamanikya is mentioned in Figure 4.

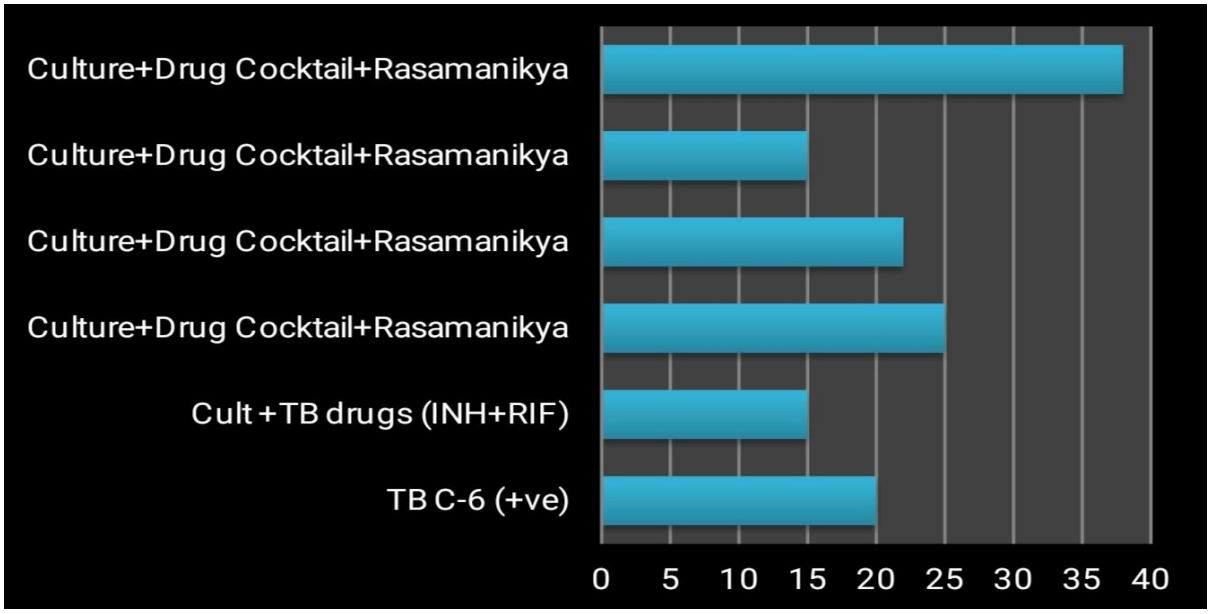

Figure 4: Based on results we found that synergistic combination of Rasamanikya with TB drugs showed enhancement of antimycobacterial activity of Rasamanikya even when used below its MIC values. Concentration of Rasamanikya (62.5mg, 46.87mg, 31.25mg, 15.62mg) were associated with significantly delay in growth times of MTB when used with MTB drugs compared to when used alone as mentioned in table 2.

Sr.no	Experimental sets	Conc	Growth mean Duration
1	TBC 6 (+ve)	10	20
2	Culture + TB drugs (INH+RIF)	50µg + 100 µg/dl	15
3	Culture + Drug Cocktail + Rasamanikya	62.5/ml	25
4	Culture + Drug Cocktail + Rasamanikya	46.87/ml	22
5	Culture + Drug Cocktail + Rasamanikya	31.25/ml	15
6	Culture +Drug Cocktail + Rasamanikya	15.62/ml	38

Table 2: Mean growth delay (in days)
****Standard MIC values of TB drugs**
***Half of the standard MIC values of TB drugs were used with culture with Rasamanikya**

DISCUSSION AND CONCLUSION

Based on the above studies, we conclude that Rasamanikya at MIC concentration 62.5/ml is unable to show significant antimycobacterial activity when comparable to MIC activity of standard TB drugs. Additionally, when also found that synergistic activity of Rasamanikya with TB drugs. Combination of TB drugs was found to significantly increase the

antimycobacterial activity Rasamanikya even when used below its MIC values.

The results of the above studies, show that Rasamanikya has potent synergistic antimycobacterial activity along with existing TB drugs. However further studies are warranted with respect to formulation, dosing & studies in larger experimental sets to justify the outcome of the present studies.

Acknowledgement

I acknowledge Shri ayurved mahavidyalya, Nagpur & personnelles of department who helped me in writing research article on antimycobacterial activity of Rasamanikya w.s.r to Tuberculosis. Special thanks to CIIMS Institute for smooth conduction of study, also I acknowledge my guide Dr. Vinod Ramteke & HOD Dr.Raman Belge sir & Dr.Rajpal Kashyap sir who always guided me in my career.

Acknowledgement: None

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

REFERENCES

1. A.P.I Text book of medicine, Shantilal j. shah, vol 1 Third Edition, 1979; p 67.
2. Am J Respir ; American Thoracic Society and Infectious Diseases Society of America. Diagnosis, treatment, and prevention of non tuberculous mycobacterial diseases. Crit Care Med 2007; 175:367– 416.
3. CDC. Controlling tuberculosis in the United States: Recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. MMWR 2005; 54 (No. RR-12).
4. Yadavaji Trikamaji, editor. Charaka Samhita of Charaka, Nidana Sthana, chapter 6, verse no.4. reprinted, Varanasi; Chowkhambha Sanskrit Series; 2008; (1);193.
5. Yadavaji Trikamaji, editor. Charaka Samhita of Charaka, Nidana Sthana, chapter 8, verse no.22. reprinted, Varanasi; Chowkhambha Sanskrit Series; 2008 ;(2);228.
6. Raviprakash Arya, K.L.Joshi, editor. Rig-Veda Samhita, Rig. 10/161 -163 Pub. Parimal Publication, Delhi, Vol- IV, 2nd revised edition, 2001;(3);94
7. Acharya Sadanand sharma, Rasatarangini, 11th ed. Motilal Banarsidas, Delhi, 5/31, p 80.
8. BrihatRasarajsundar, edited Pt.Dattaram Chowbe Yakshmaadhikhar;3rd edition Chaukhambha orientalia;Varanasi:2000 p 405.
9. Acharya Dhanduknath, Rasendrachitamni; 1st edition, Chaukhamba orientalia; Varansi; Ch5th; p65.
10. Acharya shrimadhava, AyurvedPrakasa ;Varansi,Chaukhmabavidyabhavan ;ch 2 nd p.312
11. Acharya yashodharsiddhiprada, Rasprakashsudhaker 2nd edition, Varanasi Chaukhamba orientalia 1958; Ch 4th; p 84.
12. Vinamra Sharma, Amiya k. samal,s hrutipandey et al,Characterisation of Hg based ayurvedic drug kajali; classical & contemporary approaches; current science, Vol .115 No.6;25 sept 2018.
13. Bhoyar, M.S., Khiyani, R.M. (2014). Conceptual And Pharmaceutical Review of Kupipakva Rasayana. Int. J. Ayu. Alt. Medi., 2(4):7-13
14. Puranik, G.V., Dhamankar, P.V. (1964). Ayurvidiya Aushadhikaran'part 1 & 2, Edition2nd, Mahadev Vishnushastri Puranik Publication, Dhootpapeshwar, Mumbai, chapter 19.
15. Sarkar, P.K., Mehta, N.J., Prajapati P.K. (2008), Chemistry of Kupipakwa Rasayanas –A Review.Anc. Sci. Life., XXVII (4):56-63.
16. Jim Werngren, Lisbeth Klintz and Sven E. Hoffner; Evaluation of a Novel Kit for Use with the BacT/ALERT 3D System for Drug Susceptibility Testing of Mycobacterium tuberculosis; Journal of Clinical Microbiology. 2006 Jun; 44(6): 2130–2132.
17. Fernando Rogério Pavan et. al ; Evaluation of anti-Mycobacterium tuberculosis activity of Campomanesia adamantium (MYRTACEAE);.
18. Abdella Gemechu, Mirutse Giday, Adane Worku and Gobena Ameni ; In vitro Anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis Strains; BMC Complementary and Alternative Medicine 2013.

19. Annona sylvatic; Rafaele CP Araujo, Francisco AR Neves et. al., Evaluation of the anti-mycobacterium tuberculosis activity and in vivo acute toxicity of Annona sylvatic; BMC Complementary and Alternative Medicine.
20. Renu Gupta, Bandana Thakur, Pushpendra Singh, H.B.Singh, V.D. Sharma, V.M. Katoch & amp; S.V.S. Chauhan ;Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates ; Indian Journal of Medical Research 131, June 2010, pp 809-813
21. Rajpal S. Kashyap, Amit R. Nayak, et. al. , Laboratory Investigations on the Diagnosis of Tuberculosis in the Malnourished Tribal Population of Melghat, India; Plos One, September 12, 2013
22. Gursimrat K Sandhu; Challenges and Overview of its Control Programs in India; Journal of Global Infectious Diseases. 2011 Apr-Jun; 3(2);143–150.
23. Manju Raj Purohit, Rajvi Purohit, and Tehmina Mustafa; Patient health seeking and diagnostic delay in extrapulmonary tuberculosis: A hospital-based study from Central India; Tuberculosis Research and Treatment (Hindawi), Article ID 4840561, 2019.
24. Pushpendra Singh, S. Chauhan; Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates; The Indian journal of medical research, 2010.
25. Deepthi Nair; Malini R.Capoor; Deepti Rawat; Lakshmi Srivastava; Pushpa Aggarwal; Standardization of first and second-line antitubercular susceptibility testing using BacT Alert 3D system- a report from a tertiary care centre in India; Brazilian Journal of Infectious Disease .

How to cite this article: Yogesh Madgulwar, Vinod Ramteke, Raman Belge. To evaluate antimycobacterial activity of Rasamanikya by using advance bact/alert 3-D automated liquid culture WSR to tuberculosis. *Int J Health Sci Res.* 2022; 12(8):222-229. DOI: *https://doi.org/10.52403/ijhsr.20220830*

******