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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF TAL SINDOOR

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ABSTRACT

Science is the intellectual process for using complete mental and physical resources available in order to understand, explain, quantitate and predict normal as well as unusual natural phenomena. *Rasoushadhis* (metallic and mineral preparations) are unique preparations in Ayurveda along with herbal preparations that includes *Bhasmas,* herbo-mineral preparations, and *Kupipakwa Rasayanas*. *Tal Sindoor*, a *Kupipakwa Rasayana,* is *Sagandha* (presence of Sulphur), *Sagni* (processing with heat), *Kantastha* (near the neck of the bottle) *Murchita Parada Yoga*. *Tal Sindoor* has mercury (*Parad*), sulphur (*Gandhak*) and arsenic tri sulphide (*Haratal*) as ingredients. It is indicated in all types of skin disorders (*Sarva Kushtahara*), skin problems associated with itching (*Kandu*), vitiated *Rakta Dhatu* (*Rakta dosha hara*) and other diseases of infectious origin like Abscess (*Vidradhi*), Gonorrhoea (*Upadamsha*), fever (*Jwara, Sannipataja Jwara*) at 125-250 mg (1-2 *Ratti*) dose. Anti–microbial activity of *Tal Sindoor* was conducted to evaluate drug efficacy against bacilli of gram positive, gram negative and fungus as broad spectrum antibiotic. Drug *Tal Sindoor* was tested in 2 methods i.e. Gradient plate technique and Kirby-bauer method for its anti-microbial activity against 7 micro-organisms. *Tal Sindoor* is an effective anti-microbial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*. But *K. pneumoniae* and *A. Baumannii* were resistant to *Tal Sindoor* like they are with other anti-biotics.

INTRODUCTION

Science is a gradual evolution. It is not a sudden invention, Ayurveda as a science is not an exception for it. The imperishable fundamentals of Ayurveda, which were laid down by the great sages in olden days are still applicable because of their scientific eternal background. Such fundamentals must be subjected to scientific research not only to prove its certainty but also to add something new to the existing knowledge.

The preparation of *Sindura Kalpa* can be traced back to 12th century A.D of Rasapraksha sudhakara by the name *Udayabhaskararas*, but drug *Tal Sindoor* was introduced in early 20th century with available references of preparation and indications according to Rasendra Sambhava, Basavaraajeeyam, Rasayana Sara, Siddha Bheshaja Manimala and Rasamruta. *Tal Sindoor* like *Shila Sindura*[1], is one of the mineral preparations processed by *Kupipakwa method*. *Kupipakwa Rasayana Yogas* are highly evolved pharmaceutical preparations which are boon to the ayurvedic medical field through its wide range in therapeutics. These preparations are made by amalgamating different organic, inorganic metallic and non-metallic substances with detoxified and purified mercury in specially designed fire proof bottles and special type of furnaces called *Valukayantra*. This particular chemical processes are involved in *Kuppipakwa* preparations which also bear testimony to the great chemical knowledge prevailing in ancient India. It was prepared as per the reference of the text 'Rasayana Sara' and 'Rasendra Sambhava' which has mercury (*Parada*), sulphur (*Gandhak*) and arsenic tri sulphide (*Haratal*) as ingredients. Anti-microbial study was evaluated against seven clinical types of microbes i.e., *Pseudomonas aeruginosa (P. aeruginosa)*, *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Streptococcus mutans (S. mutans)*,

Acinetobacter baumannii (*A. baumannii)*, *Klebsiella pneumoniae (K. pneumoniae)* and *Candida albicans (C. albicans)* that includes gram positive, gram negative – aerobic and anaerobic bacilli and fungal microbes.

MATERIALS AND METHODS

The materials used as ingredients[2,3] are:

1. *Parada* – Mercury
2. *Gandhak* – Sulphur
3. *Haratal* – Arsenictri sulphide

Ingredients 1-3 equal quantity by weight

4. *Kumari ras* – Aloe vera pulp (Q.S)

Other materials required for preparation of *Tal Sindoor* are:

Khalva Yantra (mortar & pestle), pyrometer, multani mitti, cloth, *Valukayantra*, *Karpura* (camphor), match box, fire wood, *Shalaka* (thin iron rod), torch, copper coin.

Materials required for anti-microbial study are:

(a) Inoculation loop, media, cotton swab, sterile discs, *Tal Sindoor*.

(b) ATCC 25922 *E. coli*, ATCC 25923 *S. aureus*, ATCC 27853 *P. aeruginosa*.

METHOD OF PREPARATION OF ANTI-MICROBIAL AGENT (*TAL SINDOOR*)

A) Purification of Ingredients: [Plate-1]

- Sulphur was purified (*Gandhaka Shodhana*[4]) with cow's milk (*Godugdha*) by *Bhudhara* method. Later *Shodhita Gandhak* was washed with warm water and collected.
- Purification of mercury (*Parada Shodhana*[5]) was done by triturating with turmeric powder (*Haridra Churna*) and pulp of *Aloe vera* (*Kumari Swarasa*). This paste was made into small circular flat pieces (*Chakrikas*) dried in shade and subjected to sublimation (*Urdhwapatana*). On self-cooling (*Svangasheetata*), *Shodhita Parada* was collected from inner surface of upper pot.
- Purification of arsenic tri sulphide (*Haratal Shodhana*[4]) was done by *Swedana* (process of purification) in *Dola Yantra* with *Benincasa hispida's* fruit pulp juice (*Kushmanda Swarasa*).

B) Preparation of *Tal Sindoor*: [Plate-1]

Mercury (*Suddha Parada*) and sulphur (*Suddha Gandhak*) are taken in equal quantity by weight are triturated to prepare *Samaguna Kajjali*[4]. Arsenic tri sulphide (*Suddha Haratal*) was triturated with *Samaguna Kajjali* to prepare *Samyaksiddha Kajjali* (uniformly triturated Kajjali). *Aloe vera* (*Kumari Swarasa*)[3] leaf pulp was added and triturated (*Bhavana*). After drying *Kajjali* was filled in glass bottle (*Kachakupi*) covered with seven consecutive layers of cloth smeared with *Multani* mud up to mouth of *Kacha Kupi*[4]. Then *Kacha Kupi* was filled with *Kajjali* and it was placed in iron vessel filled with sand (*Valukayantra*). [6]

After the entire apparatus was ready, wood was set to fire with help of camphor, following gradual heating (*Kramagni*) pattern. Pyrometer was used for recording temperature every hour. The temperature near the base of *Kacha Kupi* was maintained between 150°C – 250°C for mild heat (*Mrudvagni*), raised to 350°C – 600°C for moderate heat (*Madhyamagni*) and intense heat (*Teevragni*) up to 750°C.[7] After the stage of fumes and flames, the bottom of the bottle appears like rising Sun that is red in colour (*Udayabhaskaravarna*). After confirming with copper coin test, bottle was corked and *Teevragni* was continued for 2 hours. Later *Kacha Kupi* was left for self-cooling (*Svangasheetata*). After *Svangasheetata*, approximately equal time taken for entire *Kupipakwa*, bottle was removed from *Valukayantra*. The cloth with *Multani* was scrapped off and bottle was broken 2 inches below the collection of the *Tal Sindoor*. Later the drug was collected by tapping over the outer surface of glass bottle[7].

ANTI-MICROBIAL ACTIVITY: [Plate-2]

Antimicrobial is an agent that kills microorganisums or inhibit their growth. Anti-microbials differ not only in their action and activity but also in their distribution and metabolization and excretion by the body. Antimicrobial medicines can be grouped according to the microorganisums they act primarily against. Identification of the disease causing agent is essential for anti-microbial therapy. So drug *Tal Sindoor* was checked for its specificity with two different methods i.e by Gradient Technique and Kirby-bauer method against 7 clinical microbes[8-10].

Bacterial Strains and Culture Conditions

An ATCC 25922 *Staphylococcus aureus,* ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa* cultures were obtained from St. John's medical college, Bangalore, India. The obtained cultures were maintained on nutrient agar slants and the stock cultures were transferred at monthly intervals.

Antimicrobial Agent (*Tal Sindoor*)

The sample *Tal Sindoor* was made to fine powder. It was brownish red in colour.

I) Gradient Plate Technique

Principle

Gradient plate technique is used to isolate antibiotic-resistant bacteria mutants by exposing an agar plate containing concentration gradient of antibiotic to an inoculation of microbes to be tested.

Procedure

Agar plate was placed on a pencil to tilt one end up, so that plate was at right angle to the object

the plate is sitting on. The tilt of the plate was maintained so that the liquid doesn't quite reach to the top edge of the angled plate. Melted and cooled agar medium was poured into the plate without antibiotic and allowed it to harden. After agar was hardened in 5 minutes, the plate was set flat on the desk and medium containing the antibiotic was added. It was allowed to harden for 20 minutes. Sterile inoculation loop was used to streak microbes in a zigzag manner over the surface of the medium, taking precaution not to tear the agar. Later it was left for incubation for 72 hours. The plate was observed for the pattern of bacterial growth[8-10].

II) Kirby-bauer Method

Principle

Antibiotics are antimicrobial agents that inhibit growth of many bacteria and fungi. Diffusions of the antibiotics from the filter paper soaked in antibiotic solution results in a concentration gradient of drug. Sensitivity is measured as the zone of clearance on the lawn of sensitive bacteria. It inhibits growth of many types of bacteria and fungi. Effectiveness of antibiotics in the test is based on the size of inhibition. The zone of inhibition also depends on the diffusability of the antibiotic, the size of the inoculum, type of media and other factors.

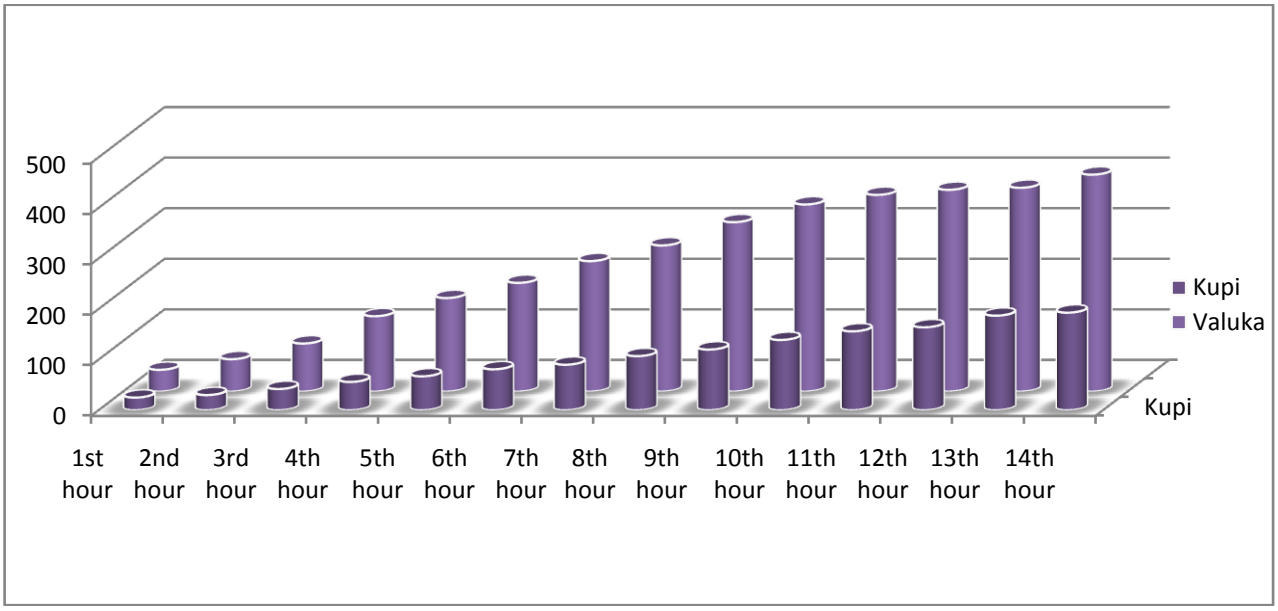
Procedure

Mueller-Hinton medium was prepared, sterilized and poured into the sterile petri plates and was allowed to solidify. Above mentioned cultures were uniformly spread on to the plates containing the media using cotton swabs. 100 mg of *Tal Sindoor* was dissolved in 1ml of methanol and 2ml of water. Sterile discs of Himedia were soaked in the suspension of *Tal Sindoor* for 10 minutes and later it was dried. The dried discs were placed on the previously swabbed petri plates. Later the plates were incubated at 37°C for 24 hours. After 24 hours of incubation the plates were checked for the formation of inhibition zone[8-10].

RESULTS

Table-1: Results of Pharmaceutical work

S.No	Drug used for process	Duration of process	Quantity before process	Quantity after process	Loss	% of yield
1.	*Parada Shodhana*	6 hrs (Sublimation)	250 g	238 g	12 g	95.2%
2.	*Gandhak Shodhana*	--	250 g	246 g	04 g	98.4%
3.	*Haratal Shodhana*	3 hrs	150 g	149 g	01 g	99.3%
4.	*Samaguna Kajjali*	36 hrs	260 g	257 g	03 g	98.8%
5.	*Kajjali (Haratal Yukta)*	06 hrs	375 g	373 g	02 g	99.4%
6.	*Tal Sindoor*	14 hrs of heating & 14 hrs of self-cooling	135 g	79 g	56 g	58.51%



Graph 1: Temperature graph during preparation of *Tal Sindoor*

Tal Sindoor was effective anti-microbial agent examined in both the methods that is Gradient Plate Technique for presence or absence of anti-microbial activity and in Kirby-Bauer activity is measured as Zones of Inhibition (Z.O.I). *Tal Sindoor* was effective against 5 microbes. *K. pneumoniae* and *A. baumannii* were resistant to *Tal Sindoor*, tested in both the methods.

Table 2: Activity of anti-microbial agent in both the methods

S.No.	Bacteria	Dosage	Activity by Gradient plate method	Z.O.I for *Tal Sindoor*
1.	*E. coli*	100 mg	+	1.6 cm
2.	*P. aeruginosa*	100 mg	+	2.0 cm
3.	*S. mutans*	100 mg	+	2.4 cm
4.	*C. albicans*	100 mg	+	3.7 cm
5.	*S. aureus*	100 mg	+	4.2 cm
6.	*K. pneumonia*	100 mg	--	--
7.	*A. baumannii*	100 mg	--	--

DISCUSSION

Murchita Parada Yogas includes *Khalvi Rasayana, Parpati Rasayana, Pottali Rasayana* and *Kupipakwa Rasayana*. *Kajjali* is a *Khalvi Rasayana* intended to remove the *Chapalatva* and *Durgrahatva* of *Parada* and potentiating it. *Kajjali Sidda Lakshnanas* (features suggestive of formation of *Kajjali*) includes *Rekhapurnata* (*Kajjali* when rubbed in between fingers it fills in furrows), *Slakshnata* (fine powder), *Nischandrata* (absence of shining particles) and *Tamra pareeksha* (*Kajjali* when rubbed over copper foil, presence of free mercury reacts to form a white streak on copper foil). These tests for *Kajjali* signify the fineness, subtleness of *Kajjali* and to strike out the chances of free mercury. Even mixing of *Haratal* by triturating is ensured for the required bonding and to prevent free molecules of mercury, sulphur or arsenic and on subjecting to heat strong bond may be formed after excess sulphur and arsenic escapes; as the form, colour and consistency transforms from fine black powder *Kajjali* to brownish red hard block *Tal Sindoor*. Triturating with liquid media plays a vital role in binding the ingredients into a single molecular form. During the process of *Kupipakwa*, *Parada* is heated steadily along with *Gandhak* and *Haratal* resulting in a very intimate bondage which may help to exhibit superior qualities compared to other formulations with same ingredients.

Valuka Yantra is specially designed for uniform and indirect heating through sand (*Valuka*) and *Valuka* is inert and may prevent the sudden rise or fall of temperatures of the *Kacha Kupi* and also may render resistance to the apparatus from variation of atmospheric temperature during *Kupipakwa* process. *Kacha Kupi* is pyro-sensitive and to sustained heat for completion of *Kupipakwa* process, *Mrith Lepana* is a prerequisite. Quantity of *Kajjali* should be filled 1/3rd volume of *Kacha Kupi* that facilitates more space for the free movement of gases and boiling of *Kajjali* during the process of *Kupipakwa*.

Tal Sindoor is an effective medicine indicated in skin disorders (*Kushtanashana Sreshtat*), skin problems associated with itching (*Kanduhara*), *Sarva Rakta Doshahara* (vitiated *Raktadhatu*) and other diseases of infectious origin like fever (*Jwara, Sannipataja Jwara*), abscess (*Vidradhi*), Gonorrhoea (*Upadamsha*).[1,2] It is effective in above conditions, might be due to the ingredients that has the following properties; *Parada* possess *Rogaghna, Rasayana, Yogavahi* properties. *Gandhak* is *Rasayana, Kushtaghna, Kandughna*, indicated in *Twak* and *Raktaghatavikara*. *Haratal* is *Katu rasa*, *Katu Vipaka, Kaphaghna, Kandughna* and *Kushtaghna*.[4] *Kumari* also is indicated in treatment of *Twak, Rakta* and *Yakritvikara*.[11]

There are number of bacteria affecting skin, respiratory tract etc. *'S. mutans'* is gram-positive, anaerobic coccus shaped bacilli. It is the leading cause of dental caries and is considered to be the most cariogenic of all of the oral streptococci. '*S. aureus'* is a gram-positive, anaerobic coccal bacilli, is both pathogenic and invasive. It produces leuko toxin and is normal flora of skin that causes infection in burns injury and discontinuation in skin. '*E. coli'* is a gram-negative, anaerobic rod shaped bacterium that is transmitted through feco-oral route from the normal flora of gut. '*P. aeruginosa'* is a gram-negative, aerobic rod shaped bacilli is present in normal flora of skin and infects in case of burn injuries. Infection of lung, urinary tract infection and kidney proves fatal. It is immune-acquired to cause pneumonia through ventilator support. '*A.baumannii*' is a gram-negative, aerobic bacterium. It is soil organism, present in hospitals and effects debilitated patients. It is resistant to many classes of antibiotics. *'K. pneumoniae*' is a gram-negative, anaerobic non-motile rod shaped bacilli. It can lead to a wild range of disease states like septicemia, Ankylosing spondylitis, soft tissue infections, skin, pharynx or gastro-intestinal tract. It is a pneumonia causing organism, second common pathogen to cause urinary tract infection next to *E. coli*. It is resistant to antibiotics like penicillin, ampicillin etc. '*C. albicans'* belongs to genus of yeast. It is present in normal flora of skin; mucosa of gastro intestine, respiratory and female genital tract. It causes infection in immune compromised conditions.

Antibiotics may have a "cidal" or "static" effect on a range of microbes. Antibiotics effective against wide range of gram-positive and gram-negative bacteria are said to be broad spectrum. *Tal Sindoor* may be grouped under broad spectrum as an attempt to understand the various aspects Acharyas tried to explain with all the indications enumerated, that are of infective origin with modern perspective. Gradient plate method indicates the anti-microbial activity and can be understood with minimum inhibition concentration. In Kirby-bauer method the antibiotic action is evaluated with zone of Inhibition.

The diameter of zone of inhibition suggests appropriate specificity of the sensitivity of *Tal Sindoor* at 100mg dose to microbes. Acharyas have specified 125-250 mg (1-2 *Ratti*) as maximum treatment dose. So 100mg of *Tal Sindoor* was taken for both the methods on all the microbes, to test its effectiveness towards the selected species with the minimal dose are within the limits *Acharyas* specified. The Gradient plate technique had shown the inhibition of microbial growth of *P. aeruginosa*, *E. coli*, *S. aureus*, *S. mutans* and *C. albicans*. This may be suggestive of the wide range of anti-biotic activity of *Tal Sindoor*. To cross check the anti-microbial activity of *Tal Sindoor* Kirby-bauer method was also selected along with Gradient plate technique. The zone of inhibition is in successive order in *E. coli*, *P. aeruginosa*, *S. mutans*, *C. albicans* and *S. aureus*. This may be suggestive of drug efficacy against *S.aureus* at the same dose i.e. 100mg. *K. pneumoniae* is often resistant to many antibiotics, including cephalosporin (e.g. extended spectrum beta-lactamase/ESBL) and aminoglycosides. *A. baumannii* is resistant to many classes of antibiotics including penicillin, chloramphenicol and often aminoglycosides. *K. pneumoniae* and *A. baumannii* are resistant to *Tal Sindoor.* Various systems of body like skin, urinary tract, respiratory system causing infectious organisms of both grams positive, gram negative, aerobic, anaerobic bacteria and fungus, had inhibition of growth which may be due to both bactericidal and bacteriostatic action, as the Kirby-bauer method showed a remarkable zone of inhibition proving the efficacy of antimicrobial agents *Tal Sindoor*.

CONCLUSION

Kupipakwa Rasayanas are unique and highly evolved pharmaceutical preparations with a wide range in therapeutics. *Tal Sindoor* is *Sagandha, Sagni, Kantastha kupipakwa Rasayana*. *Kajjali* ($Hg+S+As_2S_3$) was processed to *Tal Sindoor* with *Agni Samskara* (gradual heating process), thus altering the physico-chemical properties from that of *Kajjali*. Anti-biotic activity of *Tal Sindoor* was effective against *P. aeruginosa, E. coli, S.aureus, S. mutans* and *C. albicans* in both the methods that is Gradient Plate Technique and Kirby-bauer method. *Tal Sindoor* has no activity against *K. pneumoniae* and *A. baumannii* that is both species were resistant. Clinical efficacy of *Tal Sindoor* is a point of research. *Tal Sindoor* can be used as an effective anti-biotic as instructed and used by *Acharyas* against various infectious conditions like skin problems (*Kushtahara*) and other conditions of infectious origin like fever (*Jwara*, *Sannipataja Jwara*), Abscess (*Vidradhi*) at a dose of 125-250 mg (1-2 *Ratti*).

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Plate 1: Preparation of *Tal Sindoor*

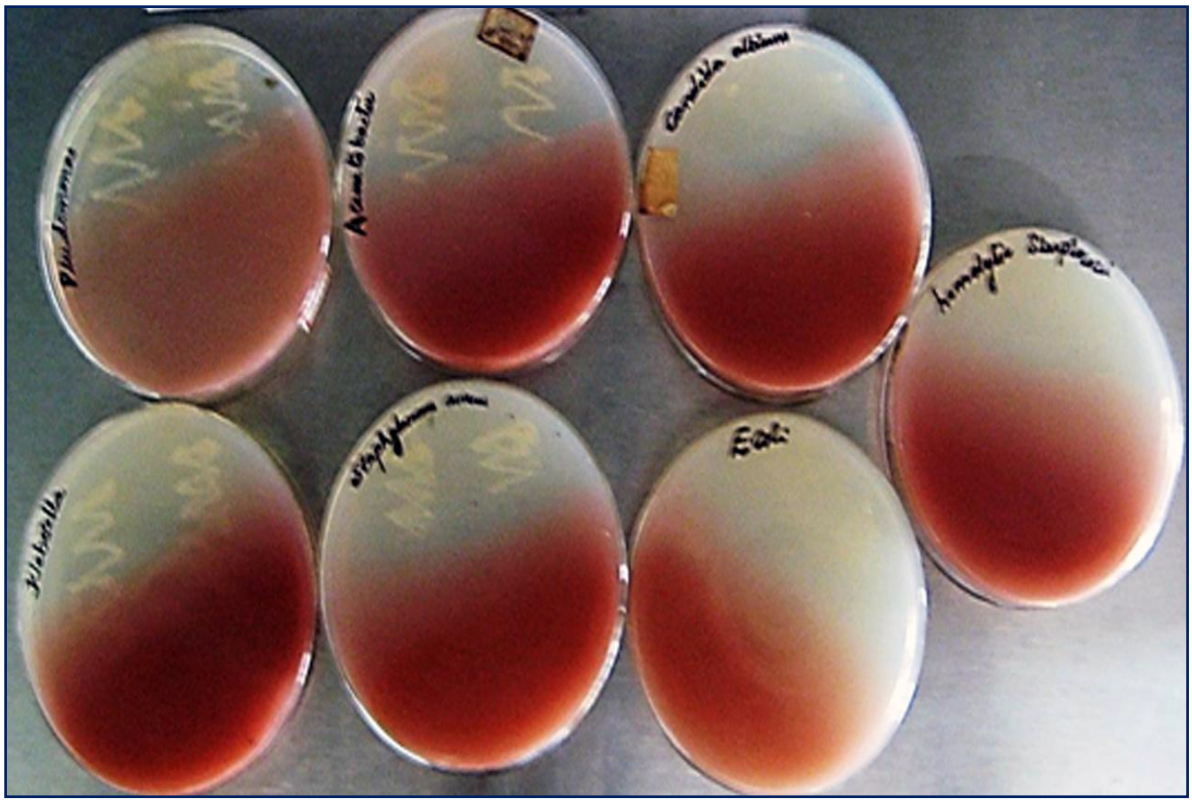

Plate 2: Gradient plate technique – Anti-biotic activity against 7 microbes