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The concept of Antimicrobial Activity in *Ayurveda* and the effect of some indigenous drugs on Gram-Negative Bacteria

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Abstract:

The diseases in ¡*yurveda* are categorized into endogenous and exogenous. Infections exercise a major part among the exogenous categories. A vivid description of infectious diseases, their pathogenesis and treatment have been documented in ayurvedic treatise. Microbes are responsible factor for infection. So to combat the microbes and their newly developing strains is a great challenge. Though, the term microbes or microbial activity have not been clearly described in the text but similar concept, their functional activities and remedies have been vividly described.

The study has been carried out for evaluation of the gram-negative activity of *āragvadha* (*Cassia fistula* Linn), *eranda* (*Ricinus communis Linn.*) and *udumbara* (*Ficus glomerata* Roxb.)

Key words: microbes, antimicrobial activity, *ayurveda*

Background :

The disease is the cumulative effect of the alter function of the *doSa*[1] and this phenomena is continued till arresting this alteration process[2]. The consequences of the altered *doSa* are replicated through the programmed dispensation and try to break the barrier of the homeostatic condition with the specific causative factors accelerates through its intensity in the occurrence of the susceptible zone[3]. The disease is produced due to the intrinsic and extrinsic factors which are endogenous and exogenous in nature respectively[4]. The profoundness of the disease process is magnified through the intense causative factors[5]. The produced disease is manifested with the different characteristics because of its varying pathogenesis and its quality of genesis.[6] The generated disease is amplified in respect to the resistance process of the body mechanism, and therefore the causative factors of the relevant diseases are classified in different ways[7].

The concept of diseases and infection in ayurveda:

The *vāta*, *pitta* and *kapha* alone, either separately or jointly produces endogenous diseases though exogenous disease may also be accompanied by the vitiation of *vāta*, *pitta* and *kapha*[8]. Ultimately the disturbances of the homeostatic condition take place for the causation of the disease[9]. The exogenous diseases at a certain stage disturb the equilibrium of *dhātus* and this disturbance in the equilibrium is also a subsidiary factor to designate as an intermediary causative factors[10]. There is an occasional overlapping in endogenous and exogenous disease. The process of developing in the secondary stage from the primary one is equally applicable to the formation of another endogenous disease in the case of the endogenous diseases and another exogenous disease in case of the exogenous diseases.[11]

The subsequent knowledge of general pathology is emphasized in the purview of microbiological concept which was evolved as the essential factors for the production of diseases and therefore this was also been incorporated in an significant way to standardize the knowledge of pathology in the prelude of quantification to make the understandable processing by the revival view of microbiology.

Therefore *nidāna* in term of external factors or *nimitta kāraNa* is to be implied here and the qualitative knowledge of compatible and incompatible diet and regime are very much important. The classified *nijaroga* and *āguntuja roga* are caused due to *sāmānya kāraNa* like *mithyā āhāra-vihāra* and *viśiSTa kāraNa* like poison, weapon, warm, insects, wild animal etc.[12]. Though all the diseases are produced due to *āsātmya indriyārthasaMyoga*, *praJjyaparādha* and *pariNAma*[13] but the classified thoughts of *sāmānya kāraNa* and *viśiSTa kāraNa* are specific to be analyzed for the production of the disease, because of behaving the immoral conduct and non-awareness of incompatible diet or avoidance of exposure to unhealthy states. In the ancient period the peoples were not susceptible to be effected by the micro-organism whereas, with the advent of times, the prevalence of common diseases were observed due to the influence of specific micro organisms and as such this was a established phenomenon of the concept of micro-organism and is regarded as the external factors for the production of diseases. The concepts of micro-organism as causative factors for the production of diseases were specified in *saMhitā* as *kRmi*. Theory of bacterial causation is established[14].

So to combat the microbes and their newly developing strains is a great challenge for us as well as a fast growing health problem. The term microbes or microbial activity have not been clearly described in the text but similar concept of microbes and their functional activities and remedies have been vividly described.

The diseases are mainly classified in two groups i.e. *nija* & *āgantuja*[15]. The term *āgantuja* is implied in broad spectrum under which trauma (external), parasite, viruses, bacteria, fungi all are been incorporated.[16] The intrinsic factor is the *sannikRSTa nidāna*, where as the extrinsic factor is the *viprakRSTa nidāna*[17]. *YakSmā* in *rājayakSmā*, *raktaja kRmi* etc. also come under microbes. The *bhUta* are considered as microbes responsible for the production of different diseases[18]. In this context *bhUta* is the intrinsic factors vitiated the *vātādi doSa*. If these microbes are identified properly then primarily the identified

microbes are to be encountered with specific drug or the growth of the different microbes may be resisted through the administration of cidal & static activity or *ghna*[19] and *hara*[20] properties correspondingly termed as antimicrobial therapy. *BhUtābhiSaGga jvara* is the result of affected systemic diseases like urinary tract infection1 respiratory tract infection, gastrointestinal tract infection etc. and accordingly the treatment procedure is to be scheduled identifying the specific pathogen.

Excessive *dāha*, *rāga* are the altered functions of *pitta* and *virecaka* drug is having the *guru*, *rUkSa* qualities, representing the properties of *kSiti* and *vāyu mahābhūta* respectively and likewise the *lekhana* action done by *vāyu* and *agni mahābhūta* and *tīkSNa* quality is beneficial to combat vitiated *kapha*[21]. *USNa* and *snigdha guNa* pacify vitiated *vāyu* in an infected stage[22]. This type of pharmacological consideration is postulated for anti-microbial activity.

Antimicrobial activity in ayurveda:

Apakarsana, *prakRtivighāta* and *nidāna-parivarjana* are the principles of treatment mentioned in context to combat the parasitic infections. But the same are also applicable for the cure of all the diseases caused by microbial infections. Therapeutically *śodhana*, *śamana* and *nidāna-parivarjana* are the respective terminologies implied in context to anti-microbial activity[23]. Śiro-*virecana*, *vamana*, *virecana* and āsthāpana are applicable for apakarSana[24]. Administration of *apāmārga*, *madana*, āragvadha[25], *eraNDa* and *udumbara* etc. are used for the said respective therapies. Simultaneously antagonist drug therapy for destruction (cidal) or limitation of the cause (static) is applied for *prakRtivighāta* and is performed through the drugs used in *krimighna* and *jvarahara* [26] etc. like *mahākaSāyas*. While administering the schedule therapies efforts should be made to avoid such causative factors which are responsible for the production of the particular disease.

The anti-microbial activity incorporates *viSaghna*, *vraNaśodhana*, *vraNaropaNa* and *kleda-pūyopaśoSaNa* activities. The ultimate aim is to arrest and encounter the infection. For these to encounter the *viSa* caused due to specific micro-organism is to be identified and accordingly the stipulated drug from *krimighna* and or *viSaghna mahākaSāya* are to be administered or considering the manifestation produced due to microorganism like *kleda*, *pūya*, *jvara*, *kaNDU*, *dāha* etc. respective *krimighna*, *kaNDUghna*, *kuSThaghna, jvarahara, śvāsahara, kāsahara, śothahara, śītapraśamana mahākaSāyas*[27] are to be used. Some groups of drugs are also used to arrest the infections caused by specific type of microorganisms characterized by different types of discharges, burning sensations, pain, redness etc. *Āragvadhādi*[28] groups destroy the organisms, alleviates itching and cleanses wound; *sālsārādi*[29] group is administered in various types of infective skin diseases; *varuNAdi*[30] group is highly effective regarding the treatment of internal abscess; *rodhrādi*[31] group arrests the diseases of female genital tract caused by different pathogens; *arkādi*[32] group encounters the parasites, skin diseases and particularly cleanses infective wound; *surasādi*[33] group is administered in respiratory infection both upper and lower tract,

infected wound and parasitic infestations; *pippalādi*[34] group is effective in acute and chronic rhinitis; *elādi*[35] group is highly effective in boils and furuncles; *vacādi* and *haridrādi* [36]. Both encounter the pathogens in the diseases like diarrhoeal disorders; *paruSakādi*[37] is advocated in urinary disorders; *priyaGgvādi* and *ambaSThādi*[38] both are useful in chronic type of dysentery and also effective in wound healing; *nyagrodhādi*[39] groups are beneficial for chronic wound arrests the infections in female genital tract; *mustādi*[40] group has positive result in female genital tract infections too; *lākSādi*[41] group is useful in infective wound and act as anti-helminthic; *tRNa-paJcamūla*[42] is highly effective in urinary tract infections. All the above conditions and or diseases which are arrested through the administration of different types of said groups of drugs are clinically caused due to infections.

AIMS and **OBJECTIVES**:

Therefore the study would be carried out with the following **aims** & **object**: —

A) To evaluate the concept of antimicrobial activity in *ayurved* in concordance with western medicine.
B) To evaluate the efficacy of *Cassia fistula* Linn, *Ricinus communis* Linn. and *Ficus glomerata* Roxb. on gram negative organism.

EXPERIMENTAL STUDY

Materials & Methods:

The following plants were selected for this study based on their medicinal use –

Plants	**Botanical Name**	**Family**	**Parts of use in the current research**
Āragvadh	*Cassia fistula* Linn.	Leguminoceae	Leaves [43]
EraNDa	*Ricinus communis* Linn.	Eurphorbiaceae	Leaves
Udumbara	*Ficus glomerata* Roxb.	Moraceae	Leaves

The leaves of the aforesaid plants are collected in spring time[44] i.e. March 2008. The experiment is carried out through following steps:

(A) **Preparation of Crude and Sterile Plant Extract**

At first the crude and sterile extract of *āragvadha, eraNDa* and *udumbara* are made according to standard process.

(B) **Making of Diffusion Disc**

Marking discs are prepared as per standard methods i.e. through autoclaving, drying and impregnating etc.

(C) **A Collection and Culture of Bacteria**

The bacteria *Escheria coli* species and *Klebsiello* species were collected separately from the stock culture of Pathology Laboratory, Department of Pathology, Institute of Post Graduate Ayurvedic Education & Research, at S.V.S.P. Hospital, Kolkata 700009; they were examined bio-chemically and morphologically.

At first 4-5 well isolated colonies of *E. coli* were selected from the stock culture of *E. coli* having same morphological type. Then at a temperature was made to touch the top of each colony with a microme wire loop, sterilised by heating through spirit lamp. Microme loop containing *E. coli* growth was transferred to a sterile tube containing 1 ml of nutrient broth medium and stirred properly for few seconds. The tube contain broth culture was allow to incubate for 2 hrs at 35^{o}C temperature in incubator until it achieved the turbidity. Likewise the culture broth of *Kleibsiella* species was also made in the above process. Now both the culture broths of bacteria *E. coli* species and *Klebsiella* species were ready for susceptibility test.

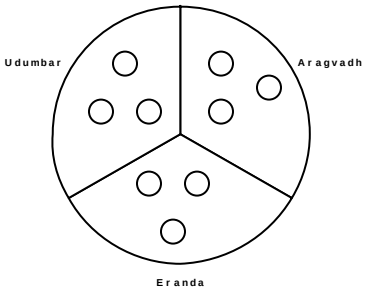
(D) **Disc diffusion susceptibility testing**

First 20 gm nutrient agar was liquefied by heating through warm water bath and poured evenly in each 4" sterile petridish divided in equal quantity. The both petridish had already been divided in three quadrants namely 'A', 'B', 'C' for *āragvadha*, *eraNDa* and *udumbara* respectively by marking lower external surface of each petridish. The liquid nutrient agar became condensed after 5 mins in normal temperatures. Now 2 inoculum suspensions of both *E. coli* species and *Klebsiella* species separately cultured were flooded evenly in both nos. (1) and (2) petridish respectively. After that previously prepared diffusion disc impregnated with crude and sterile extract of *āragvadha*, *eraNDa* and *udumbara* separately were placed in respective zone of both petridish nos. (1) and (2) by sterile forceps. Forceps were sterilised again in each time after placing one disc in respective zone by heating method with the help of spirit lamp. Then three discs were placed in triangular fashion in which two centres of the discs apart from 30 mm distance.

The discs loaded with both type of extracts were allowed to diffuse for 5 mins and both nos. (1) and (2) petridish were kept for incubation at 37^{o}C for 18-24 hrs.

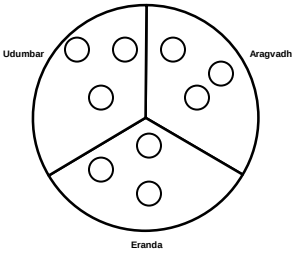
Petridish I

Bacteria – ***E. coli* sp**.



Petridish II

Bacteria: ***Klebsiella* sp**.



(E) **Observation**

At the end of the incubation, both the petridish were collected from incubator. Inhibition zone formed around the discs were measured with transparent ruler in millimetre. These studies were performed in triplicate.

In all experiments the following abbreviations is accounted

- Zone A – *Āragvadha* , Zone B – *EraNDa,* Zone C – *Udumbara*
- Zone of inhibition was measured in mm

Observation (average measured in mm is as follows)

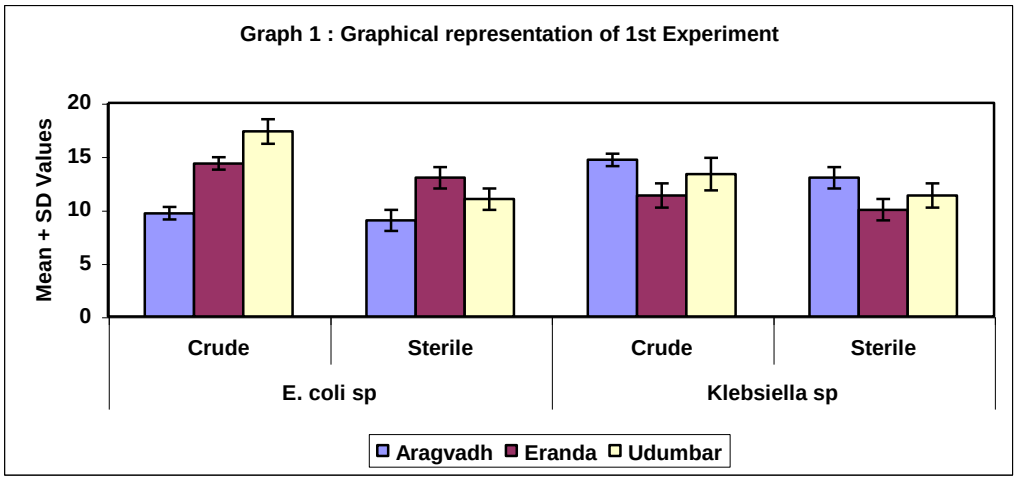
sample	Bacteria	Experiment 1		Experiment 2		Experiment 3	
		Crude	Sterile	Crude	Sterile	Crude	Sterile
Zone A	E coli. sp	9.6	9	10.3	9.6	9.6	10
Zone B	E coli. sp	14.3	13	14.3	13	13.6	13
Zone C	E coli. sp	17.3	11	17.6	11	17.3	10.3
Zone A	*Klebsiella* sp.	14.6	13	14	13	13.3	13
Zone B	*Klebsiella* sp.	11.3	10	11.3	9.6	11	10.3
Zone C	*Klebsiella* sp.	13.3	11.3	13.3	11	13.3	11

RESULT ANALYSIS:

1st Experiment:

Plant	E. coli sp.		Klebsiella sp.	
	Crude	Sterile	Crude	Sterile
	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]
Āragvadha	9.67 ± 0.58	9.0 ± 1.0	14.67 ± 0.58	13.00 ± 1.00
EraNDa	14.33 ± 0.58	13.0 ± 1.0	11.33 ± 1.15	10.00 ± 1.00
Udumbara	17.33 ± 1.15	11.0 ± 1.0	13.33 ± 1.52	11.33 ± 1.15

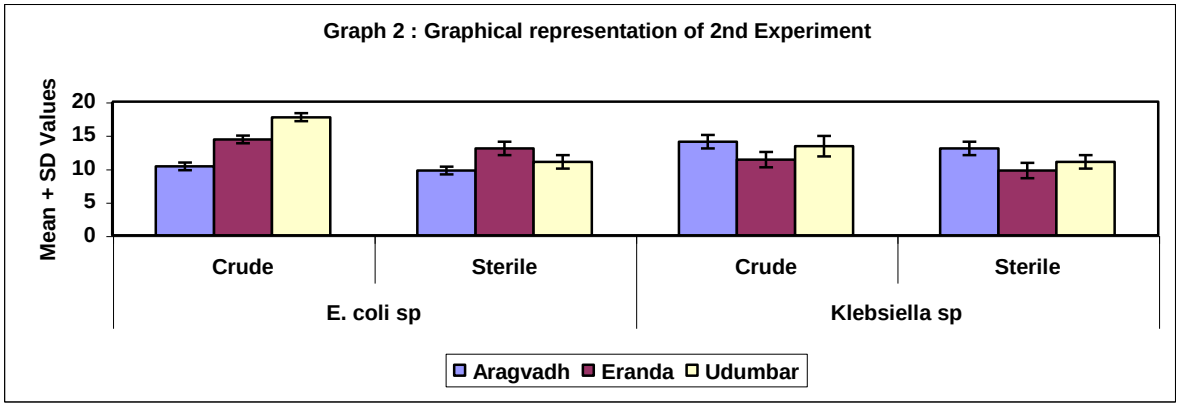
$P<0.05$



2nd Experiment:

Plant	E. coli sp.		Klebsiella sp.	
	Crude	Sterile	Crude	Sterile
	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]
Āragvadha	10.33 ± 0.58	9.67 ± 0.58	14.00 ± 1.00	13.00 ± 1.00
EraNDa	14.33 ± 0.58	13.00 ± 1.00	11.33 ± 1.15	9.67 ± 1.15
Udumbara	17.67 ± 0.58	11.00 ± 0.58	13.33 ± 1.52	11.00 ± 1.00

P<0.05

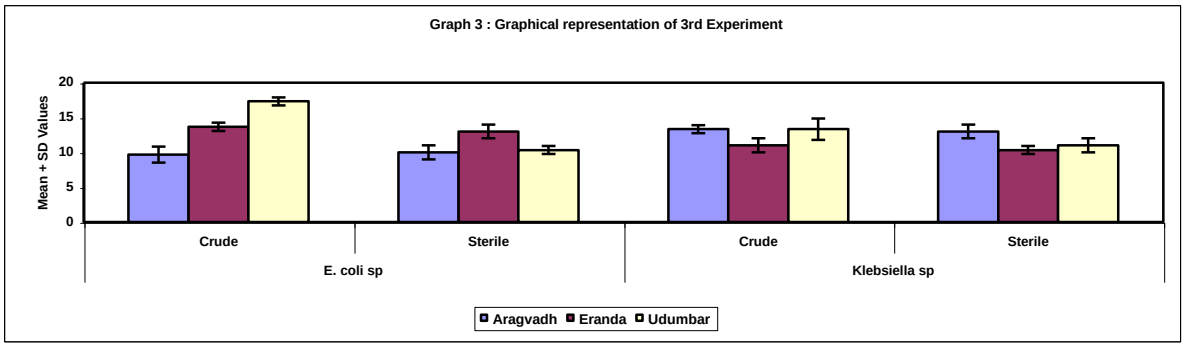


3rd

3rd Experiment:

Plant	E. coli sp.		Klebsiella sp.	
	Crude	Sterile	Crude	Sterile
	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]	(Mean ± S.D.) [mm]
Āragvadha	9.67 ± 1.15	10.00 ± 1.00	13.33 ± 0.58	13.00 ± 1.00
EraNDa	13.67 ± 0.58	13.00 ± 1.00	11.00 ± 1.00	10.33 ± 0.58
Udumbara	17.33 ± 0.58	10.33 ± 0.58	13.33 ± 1.52	11.00 ± 1.00

P<0.05



Type of extract	Bacterial	Plant	Significant
Crude	E. coli	*Udumbara*	More
Crude	Klebsiella	*Āragvadha*	More
Sterile	E. coli	*EraNDa*	More
Sterile	Klebseilla	*Āragvadha*	More

Observations and Discussion:

It is observed from the above tables

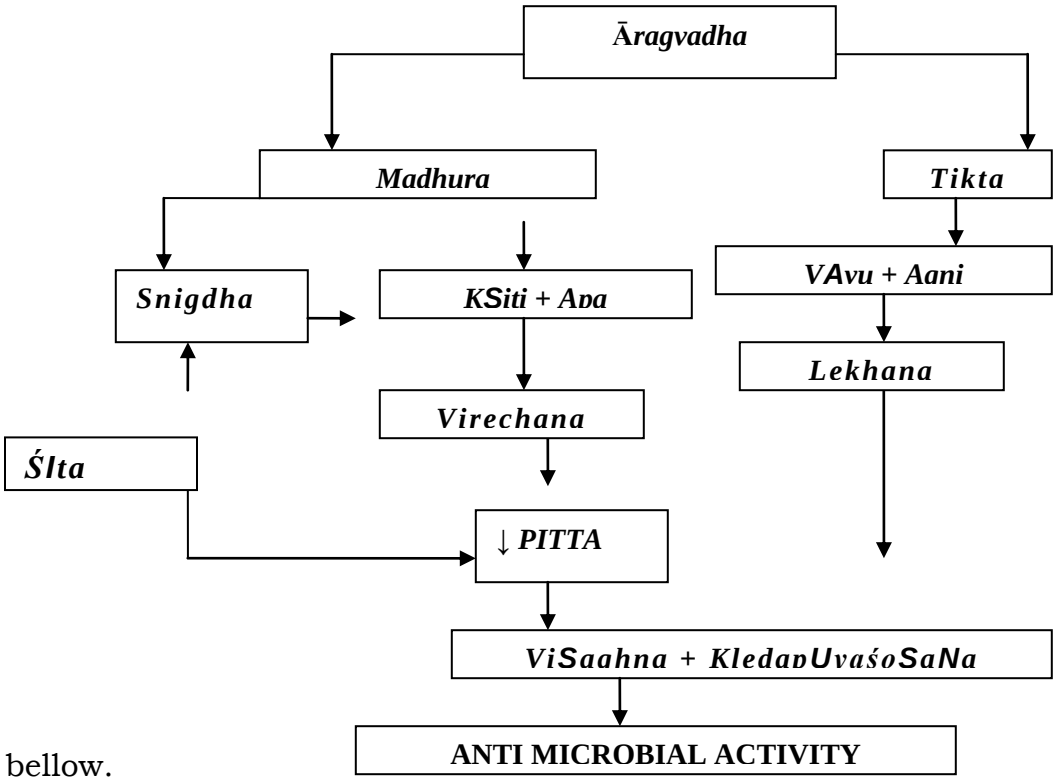
1. that the crude extract of *udumbaraa* is more effective to inhibit the colonization of E.coli, in comparison to the effect of *āragvadha* and *eraNDa*.
2. that the crude extract of *āragvadha* is more effective to inhibit the colonization of Klebsiella, in comparison to the effect of *udumbara* and *eraNDa*.
3. that the sterile extract of *eraNDa* is more effective to inhibit the colonization of E.coli, in comparison to the effect of *āragvadha* and *udumbara*.
4. that the sterile extract of *āragvadha* is more effective to inhibit the colonization of Klebsiella, in comparison to the effect of udambar and *eraNDa*.

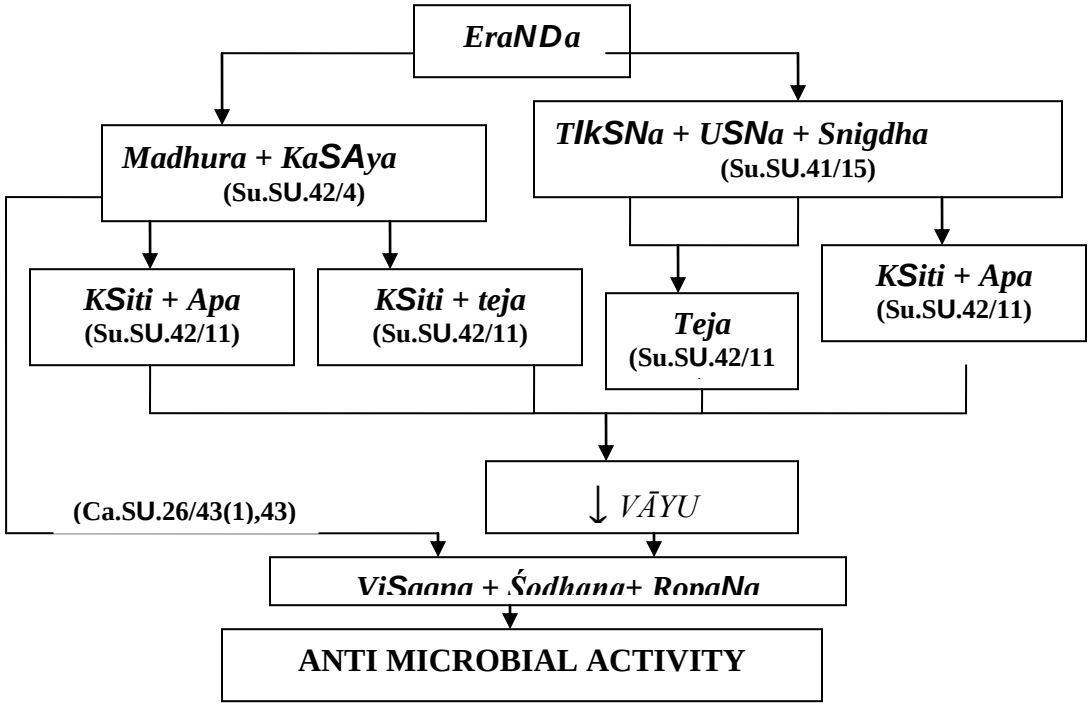
On the basis of the above observations it may be revealed that the crude and sterile extract of selected plants, named *udumbara*, *āragvadha* and *eraNDa* are effective to inhibit the zone of colonization of the micro organism {E.coli and Klebsiella}. The effectiveness of crude and sterile effect of *āragvadha* to inhibit the colonization of klebsiella, the crude extract of *udumbara* to inhibit the zone of colonization of E coli and the sterile extract of *eraNDa* to inhibit the zone of colonization of E.coli are the suggestive of antimicrobial activity. But the highest effectiveness of the crude extract of the *udumbara* in comparison to the effect of other two drugs proves the quality of the āyurvedic formulation. It may be due to that during the sterilization process of those drugs, through seitz's filter some effective constituents having antimicrobial activity are

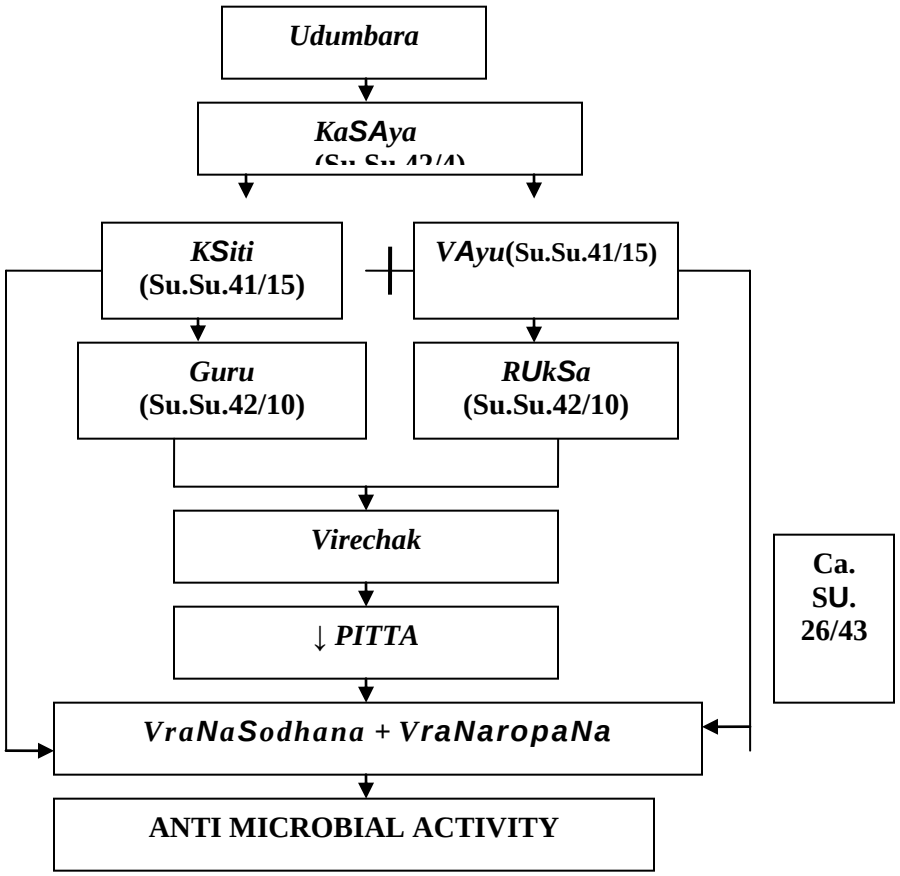
absorbed which are not profound in context to the administration of the plants in its crude form.

Schematic diagram of probable mode of action of the drugs:

All the three drugs show their antimicrobial activity which is presented bellow.




EraNDa
Madhura + KaSAya
(Su.SU.42/4)
TIkSNa + USNa + Snigdha
(Su.SU.41/15)
KSiti + Apa
(Su.SU.42/11)
KSiti + teja
(Su.SU.42/11)
Teja
(Su.SU.42/11
KSiti + Apa
(Su.SU.42/11)
↓ VĀYU
(Ca.SU.26/43(1),43)
ANTI MICROBIAL ACTIVITY


Udumbara
KaSAya
KSiti
(Su.Su.41/15)
VAyu(Su.Su.41/15)
Guru
(Su.Su.42/10)
RUkSa
(Su.Su.42/10)
Virechak
↓ PITTA
Ca.
SU.
26/43
VraNaSodhana + VraNaropaNa
ANTI MICROBIAL ACTIVITY

Conclusion:

The production of disease by microorganism is a dynamic process between an infective organism and the various defenses of the human immune system. By the indulgence in the physical contact, expired air, ingested food material with other in the same plate, sharing bed & chair, wearing used clothes, garlands and paste; *kuSTha*, *jvara*, *śoSa*, *netrAbhiSyandya* and other infectious diseases spread individuals to individuals.

The crude and sterile extract of selected plants, named *udumbara*, *āragvadha* and *eraNDa* are effective to inhibit the zone of colonization of micro organism {E.coli and Klebsiella}. The effect of the administration of the crude extract of the plant is more than the effect of the administration of sterile one in context to antimicrobial activity.

Therefore the administration of the ayurvedic drug is to be made in accordance to the principles and dosage of administration as mentioned in the authoritative text.

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