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Antioxidant & Antimicrobial Study of Ayurvedic Herbal Formulation: Kalyana Avaleha

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ABSTRACT:
Bhaishajya Kalpana is a branch of Ayurveda which mainly deals with Medicine preparation in many dosages form in which *avaleha* is one of the dosages. *Avaleha* is the Semisolid dosage form which is widely used in different age group. This research delves into the quality assessment of *Kalyana Avaleha,* a powdered formulation taken by *ghrita* which is mentioned in Ayurvedic texts for its therapeutic properties in addressing conditions related to *Swarabheda.*
Aims & Objectives: To do antioxidant and anti-microbial study of *Kalyana Avaleha*.
Material & Method: *Kalyana Avaleha* prepare and done the antioxidant activity and antimicrobial study done by using the Agar well Diffusion method against Staphylococcus aureus, Escherichia coli, Streptococcus pyogens, and Pseudomonas aeruginosa. Control substances, Gentamycin/Doxycycline for antibacterial and Fluconazole for antifungal activities, provide benchmarks for comparison. Additionally, antioxidant properties are explored through the Ferric Reducing Antioxidant Power (FRAP) assay.
Results: Notable findings in flame photometry, phytochemical and antimicrobial activities, revealing concentrations where zones of inhibition are observed. The role of electrolytes in *Swarabheda*, antibacterial actions, and antioxidant effects. The study underscores the potential therapeutic benefits of *Kalyana Avaleha,* attributing its efficacy to alkaloids, tannins, and flavonoids.

KEYWORDS: *Kalyana Avaleha*, Phytochemical, Antimicrobiology, Ayurveda, Quality Assessment.

INTRODUCTION:

Bhaishajya Kalpana is a branch of Ayurveda which mainly deals with Medicine preparation, primarily deals with *Panchavidha Kashaya Kalpana* i.e. *Swarasa, Kalka, Kwatha, hima & Phanta.* Other *upkalpana* like *Churna, Guti, Vati, Modaka, Avaleha, Sneha Kalpana, Asava-Arista* etc. are different dosage form of Bhaishajya Kalpana other than *panchavidha Kashaya Kalpana. Avaleha* is the Semisolid dosage form which is widely used in different age group. *Swarabeda[1] Utpatti* were explained as Vayu & other doshas aggravated by excessively loud speech, poisoning, reading loud, cold, and other similar causes, get localise in the channels of voice (laryngeal apparatus) and damage the voice. *Kalyana avaleha* is one such formulation in the powder form which is mentioned by Rasa Ratnakara[2] in *Swarabheda Chikitsa* and Bhaisajya Ratnavali in *Swarabheda Chikitsa*[3] and *Vatavayadhi*[4]. *Kalyana Avaleha* is mainly indicated in *Swarabheda Chikitsa* (Hoarseness/ Voice disorder) along with *Jadatwa, Gadagad, Mookhatwa* (Loss of voice) etc.,

AIMS & OBJECTIVES:

1. To evaluate the antioxidant activity of *Kalyana Avaleha.*
2. To evaluate the anti-microbial activity, *Kalyana Avaleha.*

MATERIAL AND METHODS:

The data related to *Kalyana Avaleha* is collected from Various classical text books, journals were searched and collected accordingly. Drug Procured from KLE Ayurveda Pharmacy, Khasbagh & Authenticated, and prepared in Central Research Facility, KAHER's Shri B.M.K. Ayurveda Mahavidyalaya, Belagavi, Karnataka. Further analysis regarding the formulation were done in CRF of the same college.

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Methodology:

1. Preparation of *Kalyana Avaleha*:

Haridra, Vacha, Kushta, Pippali, Sunthi, Swetha Jeeraka, Ajamoda, Yastimadhu and *Saindhava Lavana* each have taken in equal quantity and made into fine powder.

2. Antioxidants: (FRAP Assay: Ferric Reducing Antioxidant Power)[5]

Principle: The principle of this method is based on the reduction of a ferric (Fe^{+3}) 2,4,6-tripyridyl-s-triazine complex (Fe3+-TPTZ) to its ferrous (Fe^{+2}) coloured form (Fe2+-TPTZ) in the presence of antioxidants (sample and control).

Sample preparation: Plant extracts was used for antioxidant study

Standard: $FeSO_4.7H_20$

Results: are expressed in µM Fe (II)/g dry mass

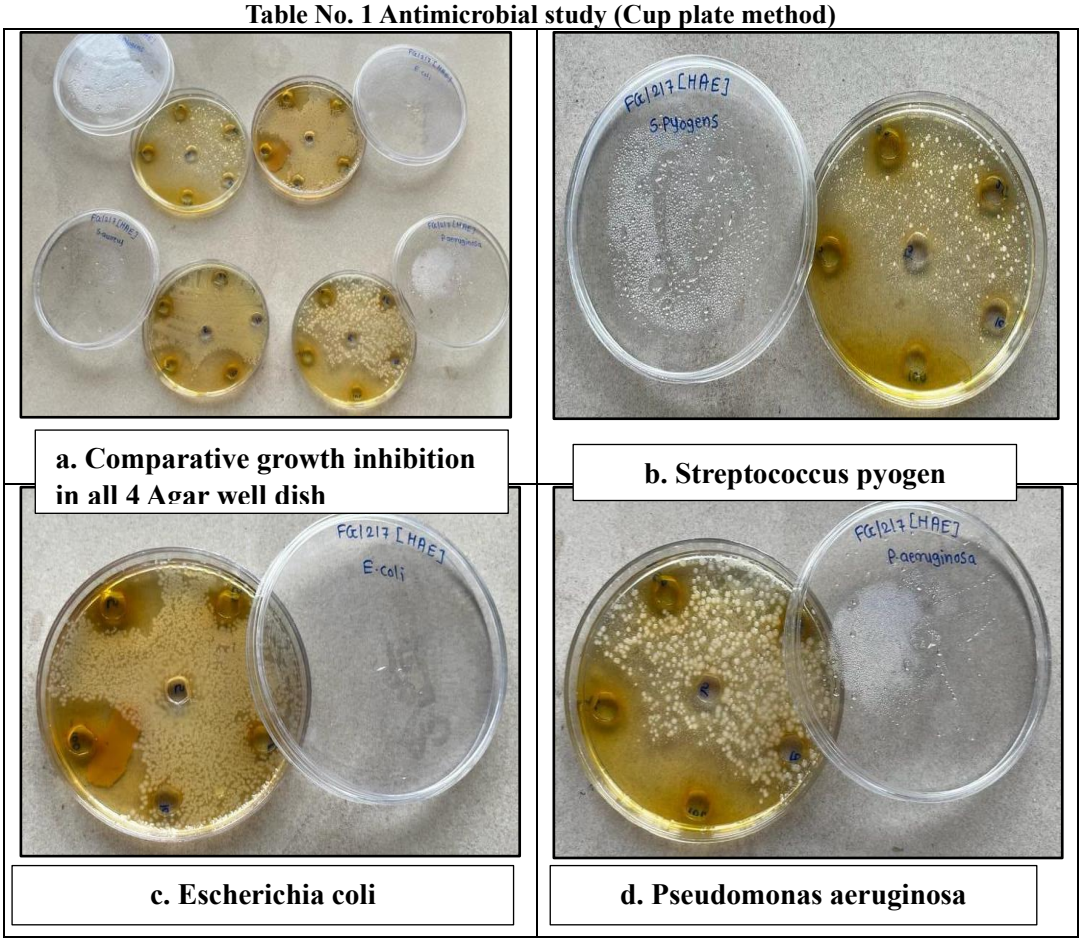
3. Antimicrobial study (Cup plate method):[6]

The antibacterial activity of the extracts is determined by using the agar well diffusion technique. Mueller-Hinton agar plates were seeded with 0.1 ml of overnight culture of Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa respectively, allowed to incubate for 24hrs. Cups were made in Petri plates using sterile cork borer (0.85 cm) and different concentrations of the extract is added into each well.

Then bacterial plates were incubated at 37ºC 24 hrs Each test compound has got six bores which corresponds to 6 concentrations that is 100, 75, 50, 25, 10, 5 for which zone of inhibition diameter and mean values are determined and recorded as diameter in mm.

a. Gentamycin/ doxycycline antibiotic was used as control: -Anti bacterial
b. Fluconazole for Antifungal

Table No. 1 Antimicrobial study (Cup plate method)



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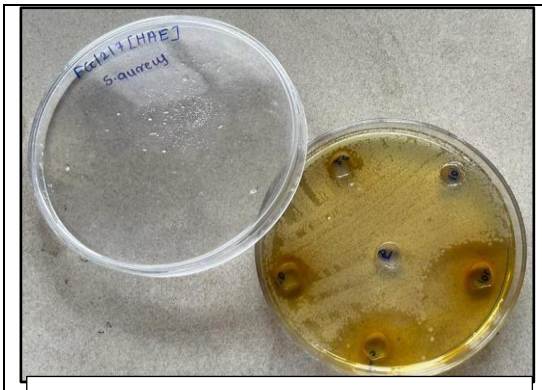

e. Staphylococcus aureus

Zone of Inhibition Observed in different pathogens with different Concentration of Kalyana Avaleha (Agar well Method)

RESULTS:

1. Antioxidants:

Y= 1.0891x + 0.022

R^2= 0.9864

WSE= 0.17±0.01 mM Fe (II)|Gram extract

ASE= 0.34±0.00 mM Fe (II)|Gram extract

2. Antimicrobial Activity (Antibacterial- activity):

Table No. Antimicrobial Activity

	1	**2**	**3**	**4**	**5**	**6**	**7**
Organism/Concentration ↓	100	75	50	25	10	5	No Drug
Staphylococcus aureus	25mm	18mm	12mm	NZ	NZ	NZ	NZ
Escherichia coli	24mm	20mm	NZ	NZ	NZ	NZ	NZ
Streptococcus pyogens	27mm	20mm	15mm	NZ	NZ	NZ	NZ
Pseudomonas aeruginosa	22mm	18mm	15mm	NZ	NZ	NZ	NZ

***** NZ= No Zone of inhibition around the drug

Reporting of the results is done as per the formation of Zone of Inhibition.

- In study conducted with the sample received there was in some concentrations zones of inhibition are seen, in some concentrations no zone of inhibition is seen on the tested organisms hence will be interpreted as ineffective against the concentration tested, corresponds to the strength of the extract.
- Result observed are exclusively for the sample received.

DISCUSSION:

Antioxidant effect:

Antioxidant compounds are needed to prevent the formation of new free radicals, and polyphenols are prominent compounds that react with free radicals. The formation of free radicals ultimately leads to oxidative stress, and the addition of antioxidant compounds can stop the further spread of Reactive oxygen species (ROS) i.e. Hydroxyl radical, superoxide, peroxide etc. Lipid peroxidation, a process in which free radicals “steal” electrons from lipids in cell membranes, leading to cell damage.[7] There are three main groups of polyphenols: flavonoids, non-flavonoids, and tannins. polyphenols are prominent compounds that react with free radicals.

Antimicrobial Activity:

The antibacterial activity of the drug was tested against four bacterial species: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*. The results are measured by the diameter of the inhibition zone (in millimetres), which indicates the effectiveness of the drug at different concentrations. The drug is most effective at higher concentrations (100%, 75%, and 50%), as demonstrated by larger zones of inhibition. Lower concentrations (25%, 10%, and 5%) show no antibacterial effect, as indicated by "NZ" (No Zone). The varying zones of inhibition suggest that *Streptococcus pyogenes* is the most susceptible to this drug, while *Escherichia coli* is somewhat less sensitive at

intermediate concentrations. This suggests that higher concentrations are necessary for efficacy, especially for organisms like *E. coli*, and that lower concentrations would not provide adequate antibacterial activity.

CONCLUSION:

This research contributes valuable insights into the quality and efficacy of *Kalyana Avaleha,* shedding light on its potential role in addressing conditions associated with *Swarabheda*. The drug showed efficacy as Anti-oxidant & Antimicrobial.

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