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DEVELOPMENT AND VALIDATION OF POLYHERBAL EAR DROPS AND ITS IN-VITRO STUDY ON ORGANISMS CAUSING OTITIS EXTERNA

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ABSTRACT

Background: *Karnasravahara Arka* is formulation preparared by fresh leaves of *Amra* (*Mangifera indica* L)*, Jambu (Syzygium cumini L.), Madhuka (Madhuca indica* J.F.Gmel*)* and *Vata* (*Ficus benghalensis* L.) which is indicated in *Karnasrava* (otitis externa) as ear drops. **Aim:** An attempt was made to develop a polyherbal ear drops *(Karnasravahara Arka)* and validate the same through analytical as well as in vitro antimicrobial study. **Methodology:** The raw drugs were authenticated through physicochemical, phyto chemical analysis and Gas chromatography–mass spectrometry (GCMS). *Karnasravahara Arka* was prepared in three batches and the samples were subjected for physico- chemical parameters and GCMS. In vitro study was carried out on organisms causing otitis externa. **Results:** *Karnasravahara Arka* can be effortlessly prepared as its ingredients are available in abundance as a natural habitat in the area of study. The ingredients of test drug show the predominance of Kashaya rasa. Preliminary phytochemical evaluation of raw drugs showed the presence of Alkaloids, Glycosides, Saponins, Flavonoids, Carbohydrates, Tannins, Proteins, and Steroids. GCMS of raw drugs indicates presence of many active principles with 15 peaks, the

13,16-Octadecadiynoic acid, methyl ester was highest peak. However, the preclinical in-vitro antimicrobial study of this formulation on organisms causing otitis externa did not show any effect.

Keywords: *Arka kalpana,* Polyherbal ear drops, *Karnasrava,* Distillation, Antimicrobial

INTRODUCTION:

Arka is one of the liquid dosage form mentioned under Panchavidha kashaya Kalpana [1]. The drugs which contain volatile active principles will get evaporated quickly if *Kwatha* is prepared with those ingredients. Prevention of the loss of active components, is the main principle behind *Arka kalpana.*

Arka in modern pharmaceutics may be compared with distillation. Distillation is a process of separating the constituents of a liquid by vaporizing and passing the vapors through a condenser, to convert them again into liquid. *Arka* is prepared by distillation of drugs which are soaked in water, with the help of distillation apparatus [2]. They are prepared in closed containers and are hence more sterile with longer self-life compared to other primary preparations of Ayurveda.

Karnasrava [3] is a most common complaint encountered in clinical practice of ENT specialty. The signs and symptoms are similar to the disease otitis externa in contemporary science. The signs and symptom are inflammation of ear, otalgia, itching, canal edema, canal erythema, and otorrhea [4].

Karnasravahara Arka [5] is a formulation preparable from leaves of *Amra* (*Mangifera indica L*), *Jambu (Syzygium cumini L.), Madhuka (Madhuca indica* J.F.Gmel*)* and *Vata* (*Ficus benghalensis* L.). As the name suggests it is indicated as an ear drop in Karnasrava. In this work an attempt was made to develop standard operative procedures for preparation of *Karnasravahara Arka* and to assess its effect through antibacterial study on organisms causing otitis externa.

OBJECTIVES:

➢ Pharmaceutical study of *Karnasravahara Arka.*

➢ To evaluate the organoleptic characters and Physicochemical parameters of the study drug

➢ To carry out the antibacterial activity of the formulation on organisms causing otitis externa

MATERIALS AND METHODS

The study was carried out in three phases.

Pharmaceutical Study: Preparation of polyherbal ear drops

Analytical Study: Development of preliminary standards for the prepared medicine by analytical study

In vitro Study: Antibacterial study on organisms causing otitis externa

PROCUREMENT OF RAW DRUGS AND AUTHENTICATION:

The Raw drugs for the preparation of *Arka* were collected from herbal garden of Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Hassan (SDMCA&H Hassan) and authenticated by the Department of Dravyaguna. The authentication was done based on morphological features, organoleptic characters and comparison with standard herbarium specimen. The preparation of Ployherbal drops (*Karnasravahara Arka*) was carried at Dept of Rasashastra and Bhaishajya kalpana, SDMCA&H Hassan. The analytical work, Total Fungal Count and Total fungal count were carried out at Dept of Dravyaguna, SDMCA&H Hassan and at Sri Dharmasthala Manjunatheshwara Center for Research in Ayurveda and Allied Sciences, Udupi. The Study was carried out in Microbiology laboratory SDMCA&H Hassan

PHASE I: PHARMACEUTICAL STUDY- Preparation of Polyherbal Ear Drops

Table 1: Raw drugs Polyherbal ear drops (*Karnasravahara arka*) [6]

Sl. No	Name of drug	Botanical name	Part used	Quantity taken
1.	Amra	*Mangiferaindica*	leaves	10gm
2.	Jambu	*Syzygiumcumini L.*	leaves	10gm
3.	Vata	*Ficusbenghalensis* L.	leaves	10gm
4.	Madhuka	*Madhucaindica*J.F.Gmel	leaves	10gm
5.	Water (w/v)			400 ml

Method of Preparation

The fresh leaves of Authenticated raw drugs mentioned above **(Table 1)** were taken and cleaned. Leaves were cut and crushed in *Khalvayantra,* soaked in round bottom flask with quantity of water sufficient enough to soak the drug for one hour. Later the remaining quantity of water was added and started heating in the *Arkayantra* (Distillation apparatus). The initial temperature was 90^0 C; once it started boiling temperature gradient was maintained between 60^0 C -70^0 C. The Initial few drops and last portion of *Arka* were discarded as it may not contain therapeutically essential substance and the process of distillation of *Karnasravahara arka* was continued till 60% of the distillate was collected. The vapors were condensed and collected in a receiver. The collected *Arka* was stored in sterile glass bottle. The medicine was prepared in three batches with same procedure.

PHASE II: ANALYTICAL STUDY: Development of Quality standards for the prepared medicine.

The procedure specified in protocol for testing Ayurveda drugs published by CCRAS was followed for analytical work. Analytical study included Physico chemical evaluation of raw drugs; Foreign matter [7], Total ash [8], Acid insoluble ash [9], Alcohol soluble extractive [10], Water soluble extractive [11], Phytochemical evaluation of raw drugs, Test for Alkaloids [12], Glycosides [13], Saponin [14], Flavonoids [15], carbohydrates [16], Tannins and Phenolic compounds [17], Proteins [18] and Steroids [19]. The prepared samples of *Karnasravahara arka* were also analysed for Organoleptic parameters and Physico chemical parameters [20] which included Volatile matter, Specific gravity, Refractive index, Determination of pH, Total Acidity, Viscosity and microbial load [21] The raw drugs and prepared samples were subjected for GC-MS [22] study.

PHASE III. ANTIBACTERIAL STUDY:

In vitro study of formulations on organisms casing otitis externa

Collection of samples

Samples of ear swabs were taken from40 diagnosed cases of otitis externa randomly selected from patients coming to Shalakya OPD. The specimen collected was cultured on Nutrient agar to develop bacteria namely *Streptococcus*.spp and *Staphylococcus*. spp. The prepared Arka extracts of different concentration (1:2, 1:5, 1:10 and 1:100) was used against isolated organisms by agar well diffusion method. The data was collected based on zone of inhibition.

Inclusion criteria:

- Age group between 16-60 yrs of both gender
- Acute otitis externa grade-1,2 and 3
- Infective Bacterial otitis externa
- Local Otitis externa
- Diffuse Otitis externa

Exclusion criteria:

- Viral/Fungal otitis externa
- Malignant otitis externa
- Seborrheic otitis externa
- Neuro dermatitis otitis externa
- Eczematous otitis externa
- Acute otitis media
- Otitis externa grade- 4
- Acute & Chronic suppurative otitis media

Nutrient agar preparation:

The 28g of nutrient agar was mixed in one litre of distilled water and allowed to dissolve completely. Sterilization was done by autoclaving at 121°C for 15 minutes. The liquid was poured into the petri dish and waited for the medium to solidify.

Working place was cleaned in laminar air flow using 70% ethyl alcohol and switch on the UV for 20 min. The sterile nontoxic cotton swab

was dipped into the inoculum suspension and rotated the swab several times with firm pressure on the inside wall of the tube to remove excess fluid. Dried surface of a MHA plate was inoculated, that has been brought to room temperature by streaking the swab three times over the entire agar surface. Lid of the dish was replaced and left it for 5 minutes. 7 equidistant wells were made on the plates with the help of sterile cork borer. 100 µl of the *Arka* extracts of different concentration (1:2, 1:5, 1:10 and 1:100) were added onto the labelled wells. All the plates were incubated at 37 ºC for 24-48 hours. After incubation period, the zone of inhibition was measured with a ruler millimeters.

OBSERVATION AND RESULTS:

The obtained *Arka* was colorless liquid which indicates the *Prashastaarka lakshana* **[23]** odor of the *Arka* possessed respective aromatic characteristic odor indicating the features of the drug.

Total Bacterial Count

The results of total Bacterial Count in samples of *Karnasravahara arka* are shown in **Table 6**. In all the three samples of *Karnasravahara arka*; Sample1, Sample2 and in Sample 3 the total bacterial count was around 7.0 X 10^4,21.7 X 10^5 and 19.0 X 10^5respectively.

Total Fungal Count (Table 7).

CFU- Colony Forming Units*TNTC-Too Numerous to Count

In all the three samples of Karnasravahara Arka; Sample1, Sample2 and in Sample 3 the total fungal count was around 5.5 x 10^4, 1.0 x 10^4 and 1.8 x 10^5respectively

Results of In-Vitro Antibacterial Study:

Based on the zone of inhibition seen in the experiment, after incubation of at 37 ºC for 24-48 hours no zone of inhibition was noted in all the plates against *Arka* extracts of different concentration **(Table 8)**.

Table 2: Observations of Pharmaceutical study

Particulars	Sample 1	Sample 2	Sample 3
Drug taken	10gm each	10gm each	10gm each
Total quantity of drug	40gm	40gm	40gm
Water (w/v)	400ml	400ml	400ml
Starting time	2.38 pm	2:44pm	2.38 pm
Boiling point	72.6^0C(2:51pm)	80^0C(2:59pm)	76.9^0C(2:56pm)
Observation of first drop of arka	At 2:52 pm	At 3:00 pm	At 2:57 pm
Heating stopped at	At 7.38 pm	At 7.44 pm	At 7.38 pm
Total duration	5 hours	5 hours	5 hours
Quantity of Arka obtained	240 ml	244 ml	238 ml

Table 3: Results showing the Physico chemical Analysis of raw drugs

Sl. No	Parameters	Amra	Jambu	Vata	Madhuka
1.	Foreign matter	2.0 %	1.8%	1.80%	2.0%
2.	Total ash	4.4 %	10.4%	7.0%	8.4%
3.	Acid –insolube ash	7.4 %	1%	2.4%	6.4%
4.	Alcohol soluble extractive	9.4 %	10.6%	9.2%	25%
5.	Water soluble extractive	14.4 %	18.4%	13.4%	64%

Table 4: Results showing thePhytochemical analysis of raw drugs

Sl No.	Parameters	Amra (*Mangifera indica*)	Jambu (*Syzigium cumini*)	Vata (*Ficus benghalensis*	Madhuka (*Madhuca indica*)
1.	Alkaloids	Present	Present	Present	Present
2.	Glycosides	Present	Present	Present	Present
3.	Saponins	Absent	Present	Present	Present
4.	Flavonoides	Present	Present	Present	Present
5.	Carbohydrates	Present	Absent	Present	Absent
6.	Tannins	Present	Present	Present	Present
7.	Proteins	Present	Absent	Present	Absent
8.	Steroids	Absent	Present	Absent	Present

Table 5: Results showing the Physico chemical parameters samples of *KarnasravharaArka*

Parameter	Results n = 3% w/w			
	Karnasravaharaarka-Batch -1	Karnasravahara arka-Batch -2	Karnasravahara arka -Batch -3	Average
Volatile matter (%)	0.12	0.10	0.10	0.11
Specific gravity	1.0043	1.0040	1.0042	1.0042
Refractive index	1.33256	1.33254	1.33254	1.33255
pH	7.0	7.0	7.0	7.00
Total acidity	0.015	0.014	0.014	0.014
Viscosity	0.9634	0.9634	0.9632	0.9633

Table 6: Results showing the Total Bacterial Count of *Karnasravaharaarka Samples*

Sl. No	Dilutions	Number of Colonies (NOC)		CFU/ml
Sample-1	$1/10(10^1)$	>300	>300	TNTC*
	$1/100(10^2)$	>300	>300	TNTC*
	$1/10000(10^4)$	8	6	7.0×10^4
Sample-2	$1/10(10^1)$	>300	>300	TNTC*
	$1/100(10^2)$	>300	>300	TNTC*
	$1/10000(10^4)$	208	226	21.7×10^5
Sample-3	$1/10(10^1)$	>300	>300	TNTC*
	$1/100(10^2)$	>300	>300	TNTC*
	$1/10000(10^4)$	176	204	19.0×10^5

CFU- Colony Forming Units *TNTC-Too Numerous to Count

Table 7: The results showing the Total fungal count in samples of *Karnasravahara arka*

Sl. No	Dilutions	Number of Colonies (NOC)		CFU/ml
Sample-1	$1/10(10^1)$	>300	>300	TNTC*
	$1/100(10^2)$	>300	>300	TNTC*
	$1/10000 (10^4)$	5	6	5.5×10^4
Sample-2	$1/10(10^1)$	>300	>300	TNTC*
	$1/100(10^2)$	>300	>300	TNTC*
	$1/10000(10^4)$	1	1	1.0×10^4
Sample-3	$1/10(10^1)$	>300	>300	TNTC*
	$1/100(10^2)$	>300	>300	TNTC*
	$1/10000(10^4)$	21	15	1.8×10^5

Table 8: Results showing the effect of *Karnasravharaarka* samples on organisms at different concentrations

Concentrations	*Staphylococcus*.spp	*Streptococcus*.spp
1:2	Resistant	Resistant
1:5	Resistant	Resistant
1:10	Resistant	Resistant
1:100	Resistant	Resistant

(Note: Resistant - No zone of inhibition observed)

DISCUSSION

The *Karnasravahara Arka* was prepared in three batches and the same procedure was followed during preparation. The 60% of distillate is collected probably the maximum active principles would be extracted by this volume. Further attempts may tend to char the contents and hence avoided. Taste of *Karnasravahara Arka* possessed *Kashaya rasa* may be because of the *Amra* and *Vata* leaves. The obtained *Arka* was colorless liquid which indicates the *Prashasta arka lakshana,* odor of the *Arka* possessed respective aromatic characteristic odor indicating the features of the drug.

The leaves of *jambu* possessed more ash value. High ash content can be an indication of presence of calcium, aluminium, manganese in the drug. Acid insoluble ash was more in *Amra* leaves compared to other leaves. It indicates the presence of silicates, oxalates, carbonates and phosphates in more quantity. Here in both the water soluble and alcohol soluble extractive values were more in *madhuka* leaves. i.e. 25%and 64% respectively. The less extractive value indicates the addition of exhausted material, adulteration or improper processing during drying or storage of drugs. The Phytochemical evaluation of raw drugs revealed the Presence of Alkaloids, Glycosides, Flavonoides,

Tannins, carbohydrates and Proteins, steroids, saponins were present in the fresh leaves.

The Alkaloids possesses the anti-inflammatory anaesthetics and cardioprotective properties [24]. Saponins possess a wide range of biological activities, such as anti-inflammatory, antibacterial, analgesic, antitumor, antiviral, immune regulation [25] properties. Flavonoids have known for their antimicrobial, antiviral, antiangiogenic [26] activity. The Steroids possesses the anti-inflammatory activity [27]. Plant tannins are hydroxyl groups and possess the antibacterial properties, which are positively correlated with antioxidant properties and mainly has effect on streptococcus and staphylococcus organisms [28]. Hence phyto constituents present in these four leaves act as anti- inflammatory and antibacterial so the *Arka* (ear drops) prepared from these drugs act as Anti-inflammatory, Analgesic and Antibacterial which are needed in the treatment of *Karnasravaha* (Otitis externa).

The pH of *Arka* samples was 7.0 suggestive of alkaline in nature; the efficacy, absorption, irritability depends on pH of a drug. If the medicine is very acidic or very alkaline it will cause irritation to the tissues. It also influences on the rate of oxidation.The presence of dissolved substances in the samples changes

the value of specific gravity. As it is a water soluble extract preparation the specific gravity of Arka was in all the samples which was similar to specific gravity of water. Viscosity of all the three *Arka* samples (Sample 1,2 and 3) were 0.9634, 0.9634 and 0.9632 respectively which was similar to that of water as it was a distillate of water. The refractive index value indicates the concentration of active principles dissolved in a solvent as it was a distillate it would only have water soluble active principles along with volatile principles extracted from the fresh leaves. The refractive index value was similar in all the three samples. Volatile matter indicates the volatile active principles in the formulation. In fact, *Arka* is a formulation where both thermo stable and thermo liable principles are seen in the final product. However, in the present study the percentage of volatile matter was meager and was also supported by the findings of GCMS. The report of GCMS of raw drugs conveyed that there are multiple volatile compounds in the powder of raw drugs out of which Methyl-3-phenoxybenzoate, 13,16-Octadecadiynoic acid, methyl ester, 4-Cyclo propyl carbonyl oxy tetradecane, Pterin-6-carboxylic acid is the highest peak is the compound which covered maximum area of the graph depicting the highest concentration in the sample. In GCMS study of prepared all the three prepared samples of *Karnasravahara arka* no peaks were observed in the chromatogram probably the voltile principles present in the raw drugs may have been lost during the preparation of Arka.

The total bacteria count (TBC) and the total fungal count of a sample is a quantitative estimate of the number of microorganisms present in a sample. It is basically, the number of colonies counted on a petri dish. This measurement is represented by the number of colony-forming bacterial units (CFU) per gm/milliliter in the sample. In the three samples of *Karnasravaharaarka*; Sample1, Sample2 and in Sample 3 the total bacterial count was around 7.0×10^4, 21.7×10^5 and 19.0×10^5 respectively which was more in sample 2. The total fungal count was around 5.5×10^4, 1.0×10^4 and 1.8×10^5 respectively which was more in sample1. These observations may be because of using fresh raw drug without drying or sterilisation. Either of them cannot be done as per the SOP of pharmaceutical study.

In the present vitro study, the antibacterial activity of *Karnasravahara arka* was analyzed against organisms causing otitis externa; *Staphylococcus*. spp and *Streptococcus*. spp. Ear swab samples were collected from patients for this study. It is observed that the patients suffering from otitis

externa were in the age group of 25 to 60 years. There is no age specification for the occurrence of the otitis externa. It was observed that the incidence of occurrence of otitis externa was more in males than in females probably males do intense work outside the house where they are exposed more to external environment.

The assessment of Anti-bacterial activity of *Karnasravahara Arka* was done at a different concentration; 1:2, 1:5, 1:10 and 1:100 to understand its effectiveness. The Anti-bacterial study was done by Agar well diffusion method. The trial samples were used to check the activity against the organisms. It is observed that both the organisms were resistant and there was no zone of inhibition observed in any of the concentrations. This may be because the concentration of the *Arka* would have been insufficient to show any activity as it is distillate where volatile principles are extracted in a water media. The lowest concentration of sample that will inhibit the visible growth of a microorganism is called as Minimum Inhibitory Concentration (MIC). In this study, it was not possible to determine the MIC.

CONCLUSION

Karnasravahara arka can be effortlessly prepared as its ingredients i.e. leaves of *Vata, Amra, Jambu* and *Madhuka* are available in abundance as a natural habitat in the area of study. The ingredients of test drug shows the predominance of Kashaya rasa. Preliminary phytochemical evaluation of raw drugs showed the presence of Alkaloids, Glycosides, Saponins, Flavonoids, Carbohydrates, Tannins, Proteins, and Steroids. Gas Chromatography and Mass Spectroscopy of raw drugs indicates presence of many active principles with 15 peaks, the 13,16-Octadecadiynoic acid, methyl ester was highest peak. The organoleptic and physicochemical properties of *Karnasravahara arka* generated in this work can be considered as preliminary standards for the formulation. The study formulation *Karnasravahara arka* (Polyherbal eardrop) did not show any effect during in vitro antimicrobial study on organisms causing otitis externa.

Acknowledgement

BIBLIOGRAPHY:

[1] Ravana, Indradev Tripathy. Arkaprakasa. 2nd ed. Varanasi. Chowkamba; 2006, P.09

[2] CCRAS. The Ayurvedic Formulary of India. 2nded. Newdelhi: Govt of India,

Health and family welfare;2003 Part1.Vol 1.P.27

[3] Acharya Yadavaji Trikamji,editor. Nibandha sangraha Commentary of Sree Dalhanacarya. 7th ed. 3-5. Vol. 20. Varanasi: Chaukhamba Orientalia; 2002. Sushruta, Sushruta Samhita, UttaraTantra

[4] Bhargava KB. A short textbook of ENT. Diseases. P.L. Dhingra – Diseases of Ear, Nose & Throat. John Jacob Ballenger- Diseases of Nose, Throat & Ear.

[5] Dr. Indradeva Tripathi. Arkaprakash (Hindi teeka and notes) 1/85; 3rd ed. Varanasi; Chowkhamba krishnadas academy; 2011. p. 115

[6] Dr. Indradeva Tripathi. Arkaprakash (Hindi teeka and notes) 1/85; 3rd ed. Varanasi; Chowkhamba krishnadas academy; 2011. p. 115

[7] The Ayurvedic Pharmacopoeia of India (API), Part-I, Appendices - 2, First Edition, Government of India, Ministry of Ayush, Published by Pharmacopoeia Commission for Indian Medicine &Homoeopathy, Ghaziabad 2016; 113-114

[8] The Ayurvedic Pharmacopoeia of India (API), Part-I, Appendices - 2, First Edition, Government of India, Ministry of Ayush, Published by Pharmacopoeia Commission for Indian Medicine &Homoeopathy, Ghaziabad 2016; 113-114

[9] CCRAS. Laboratory Guide for the Analysis of Ayurveda and Siddha Formulation. 1st ed. New Delhi. Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. of India; 2010.p.28

[10] CCRAS. Laboratory Guide for the Analysis of Ayurveda and Siddha Formulation. 1st ed. New Delhi. Dept. of AYUSH, Ministry of Health and Family Welfare Govt. of India; 2010.p.30.

[11] CCRAS. Laboratory Guide for the Analysis of Ayurveda and Siddha Formulation. 1st ed. New Delhi. Dept. Of Ayush Ministry of health and Family Welfare Govt. of India; 2010.p.29.

[12] Shah BN. Textbook of pharmacognosy and phytochemistry. Elsevier India; First edition 2010. p. 189

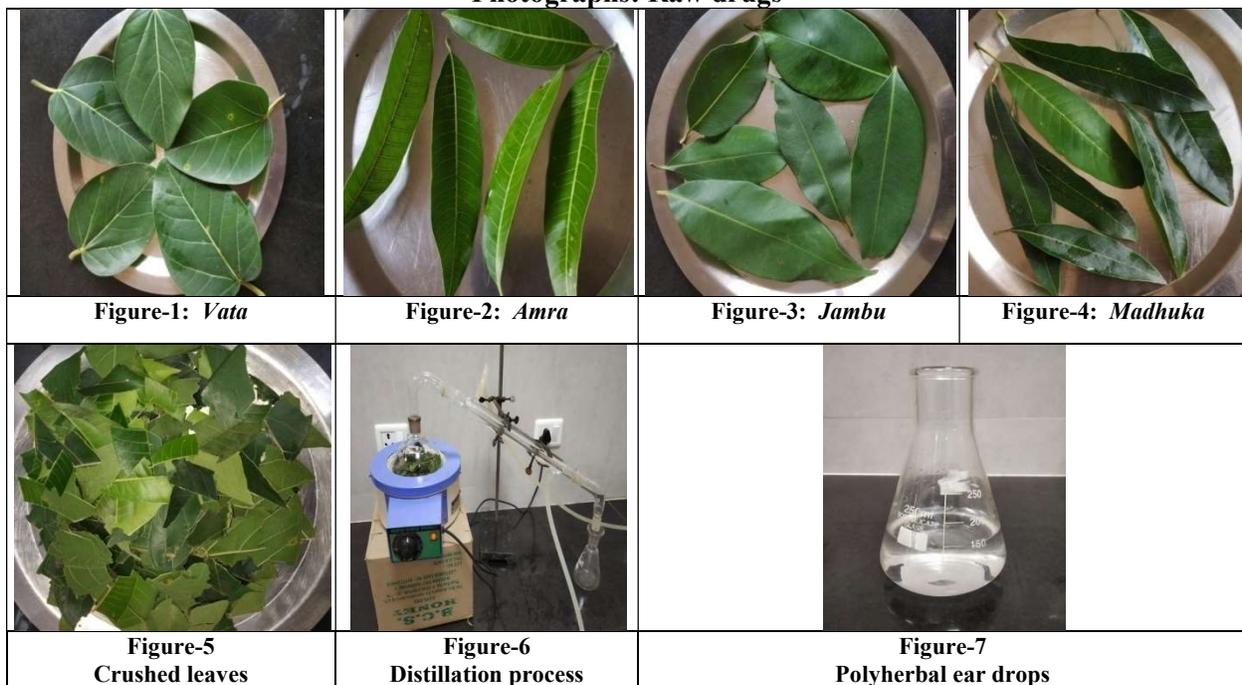[13] Shah BN. Textbook of pharmacognosy and phytochemistry. Elsevier India; First edition 2010. p. 233

[14] Shah BN. Textbook of pharmacognosy and phytochemistry. Elsevier India; First edition 2010. p. 233

[15] Shah BN. Textbook of pharmacognosy and phytochemistry. Elsevier India; First edition 2010. p. 234

[16] Khandalwal KR, Sethi VK. Practical Pharmacognosy techniques and experiments. Nirali Prakashan. Twentieth edition; 2008:p 25.0.

[17] Khandalwal KR, Sethi VK. Practical Pharmacognosy techniques and experiments. Nirali Prakashan. Twentieth edition; 2008:p 25.6.

[18] Shah BN. Textbook of pharmacognosy and phytochemistry. Elsevier India; First edition 2010. p. 386

[19] Khandalwal KR, Sethi VK. Practical Pharmacognosy techniques and experiments. Nirali Prakashan. Twentieth edition; 2008:p 25.3.

[20] Government of india, Ayurvedic Formulary of India, 1st ed. New Delhi: the controller of publications, civil Lines; 2000.

[21] Al-Mariri A, Safi M. *In vitro* antibacterial activity of several plant extracts and oils against some

gram-negative bacteria. *Iran. J. Med. Sci.* 2014;39(1):36–43.

[22] CCRAS. Laboratory Guide for the Analysis of Ayurveda and Siddha Formulations. Firstedition. New Delhi. CCRAS Department of AYUSH, Ministry of Health and Family Welfare; 2009:p.93.

[23] Ravana, Indradev Tripathy. Arkaprakasa. 2nd ed. Varanasi. Chowkamba; 2006, P.14

[24] Kurek J. Alkaloids—Their Importance in Nature and Human Life. Intech Open; London, UK: 2019. Introductory Chapter: Alkaloids.

[25] Hassan H.S., Sule M.I., Musa A.M., Musa K.Y., Abubakar M.S., Hassan A.S. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. Afr. J. Tradit. Complementary Altern. Med. 2012;9:250–255. doi: 10.4314/ajtcam.v9i2.10.

[26] Zhao, L.; Yuan, X.; Wang, J.; Feng, Y.; Ji, F.; Li, Z.; Bian, J. A review on flavones targeting serine/threonine protein kinases for potential anticancer drugs. Bioorganic Med. Chem. 2019, 27, 677–685.

[27] Snehal S. Patel, Jignasha K. Savjani- Systematic review of plant steroids as potential antiinflammatory agents:

Current status and future perspectives, The Journal of Phytopharmacology 2015; 4(2): 121-125.

[28] Wijesinghe W, Ahn G, Lee WW, Kang MC, Kim EA, Jeon YJ. Anti-inflammatory activity of phlorotannin-rich fermented Ecklonia cava processing by-product extract in lipopolysaccharide-stimulated RAW 264.7 macrophages. J ApplPhycol. (2013) 25:1207–13.
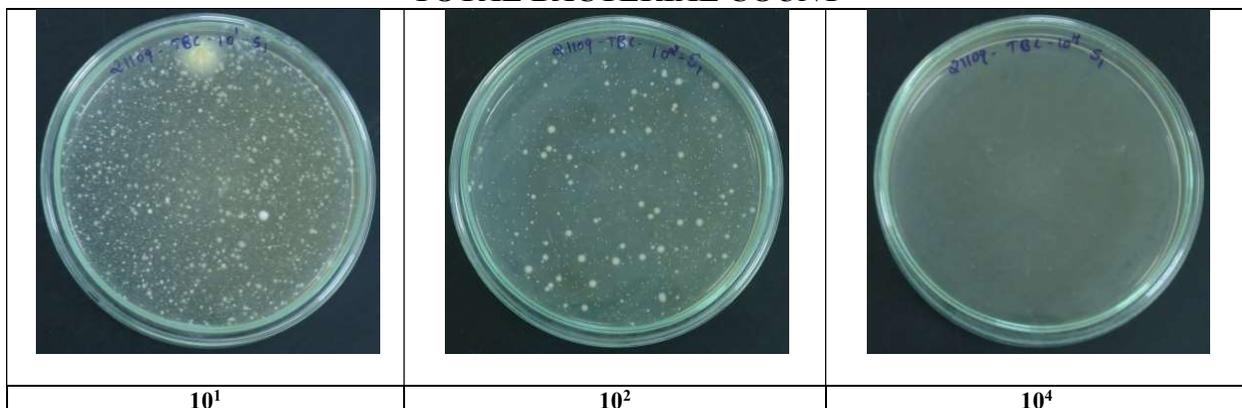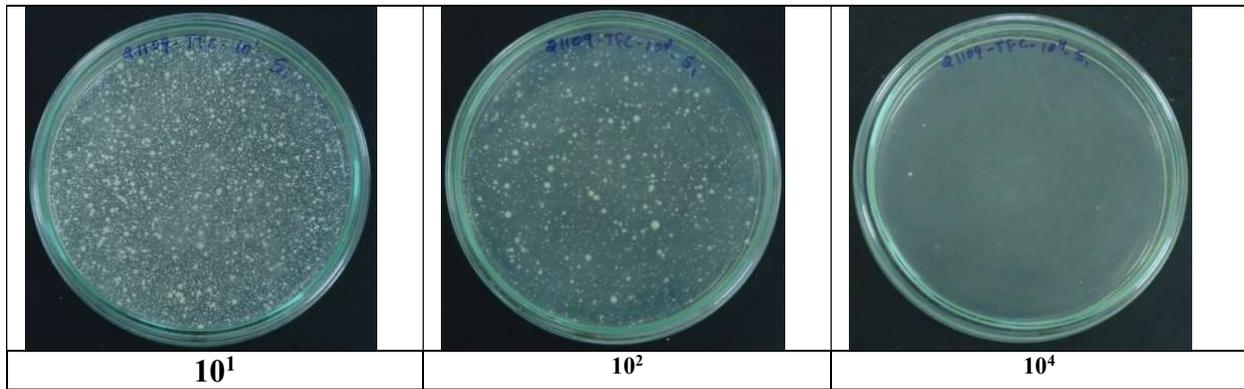
Photographs: Raw drugs

Figure-1: *Vata*	**Figure-2:** *Amra*	**Figure-3:** *Jambu*	**Figure-4:** *Madhuka*

Figure-5 **Crushed leaves**	**Figure-6** **Distillation process**	**Figure-7** **Polyherbal ear drops**

TOTAL BACTERIAL COUNT

10^1	10^2	10^4

Figure 8: Total Bacterial Count of Karnasravahara arka

TOTAL FUNGAL COUNT

| 10^1 | 10^2 | 10^4 |

Figure 9: Total Fungal Count of Karnasravahara Arka