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A COMPARATIVE STUDY OF PHYTOCHEMICAL AND MEDHYA KARMA (MEMORY ENHANCING ACTIVITY) OF NATURALLY GROWN VACHA (ACORUS CALAMUS LINN.) AND VACHA (ACORUS CALAMUS LINN.) PROPAGATED BY TISSUE CULTURE IN MICE – A PROTOCOL

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ABSTRACT

Background: Some medicinal plant has special requirement for successful cultivation. The plant species are increasingly rare due to over exploitation by various local tribes of medicine and pharmaceutical industries. Number of this medicinal plant is now facing red listed category. Many medicinal plants species at high risk. Plant tissue culture technology has been widely used for large number of plant multiplication, plant propagation and treated diseases. In short period of time, nowadays many Medicinal drugs are not available. So, to overcome this problem we should accept different method by which drug may be available for treated disease. In such a scenario like tissue culture, artificial propagation will be use. In comparison to demand there is less cultivation of *Vacha (Acorus calamus Linn.)* This is making the plant endangered and research on MEA as tissue culture in *Vacha (Acorus calamus Linn.)* are knee of interest as these drugs they are capable of improving memory according to Ayurveda. Thus, comparative study of *Vacha (Acorus calamus Linn.)* collected from natural habitat and grown from different part of plants through tissue culture was not done as well the study of these

two-drug analysis. **Aims and Objectives:** Comparative study of Phytochemicals and *Medhya Karma* (Memory Enhance activity) of naturally grown *Vacha (Acorus calamus Linn.)* and *Vacha (Acorus calmus Linn.)* propagated by Tissue Culture in Mice. **Methodology:** Procedure in this examination 2 sample of *Vacha* (*acorus calamus Linn.)* i.e., naturally grown and tissue cultured *Vacha (Acorus calamus Linn.)* will be separated and it will be divided into 36 mice in 6 group (6 mice in each). This sample will be carried out to evaluate Memory Enhance Activity by two methods Morris water maze and elevated plus maze. **Expected Results:** Result will be noted during physical and chemical analysis and during the study. **Conclusion:** Conclusion will be drawn on the basis of observation at the end of stud**y.**

KEYWORDS: *Vacha* (*Acorus calamus Linn*.), Tissue culture technique, Memory enhancing activity.

INTRODUCTION

Ayurveda is the science of life with its antiquity has embraced new drug into its Materia medica during the course of time. Anything and everything in this universe can be used as medicine provide on has vision to use it in a proper way and in appropriate condition for desired effect.[1] For these purpose drug plays an important role where the drug may be used as herbs, mineral and any kind of substance *Dravya* literally means composition from by the *Panchamahabhuta*.

In *Dravyaguna* is a science *Dravya* has kept on the priority all other basic principle of *Dravya* this is because *Dravya* has shelter house to all the components like *Rasa, Guna, Vipak, Viraya* and *Prabhav.*[2] So, The *Acharya* has specified the importance of *Dravya* has *Vyavasthitatva, Nityatvyatv, Swajati Awastha, Panchendiyagrahan, Ashrayatva, Aarambhasamarthya, Shastrapramanya, Kramapekshittva, Ekadeshsadyatvya, Taratamayoga anupalabdhi, Vikalpasamarthya* and *Pratighatsamarthya* all and also *Acharya* had told that now should drug.[3]

It should have verity. It should prepare many types of all *Kalpana* and has many *Yogas*. For maintenance of healthy life *Ayurveda* has described the basic principles like *Tridoshas* and *Panchamahabhutas*, which states all the substances present in the universe are made up of *Panchamahabhuta* (*Akash, Vayu, Tej, Jala, Prithvi*) including *Tridoshas, Dravyas* and body constitutes. This has its importance in treatment and maintaining health. Aim of *Ayurveda* is to maintain the health of healthy and to treat the disease or ill person.[4] To procure this motto

of *Ayurveda*, we have to depend on *Dravyas*. To know the *Dravyas* and principle of *Panchamahabhuta* we should first know about the branch of *Ayurveda* that refer to it.

Ayurvedic plants are the main source of living being i.e., direct and indirect in life. In *Ayurveda* plants are used for great extent because they having medicinal property. Such category medicinal plant is diminishing day by day, whose conservation and protection are the needs of era. New techniques are adopted for the same.

Some medicinal plant has special requirement. This has to meet their successful cultivation. The plant species are increasingly rare due to over exploitation by various local tribes of medicine and pharmaceutical industries.[5] The rate of replantation wild source of many such medicinal plant species has out patched regeneration capacity with sad results. Numbers of this medicinal plant is now facing threats of extinction and are enlisted under red listed category. As medicinal plant received increased commercial and scientific attention, there is increasing pressure on wild plant population that is why most of medicinal plants are harvested. Over harvesting place are many medicinal plants species at high risk. Commercial exploitation has also sometime led to medicines becoming unavailable.

Plant tissue culture technology has been widely used for large number of plant multiplication, plant tissue culture techniques are major importance in area of plant propagation and diseases of secondary metabolites. A single medicinal explant can be multiplied in in several thousand medicinal plants in short period of time and space under the controlled conditions, irrespective seasons and weather on year-round basis. Endangered species have been successfully grown and conserved by micro propagation method.[6]

The literal meaning of plant tissue culture i.e., explant culture. It provides a vitro model of tissue which can be easily manipulated and analysed. Tissue culture is the militancy of plant cells or sterile organ environmentally and nutritionally supportive condition. In commercial setting, tissue culture is primarily used plant propagation which refers to the production of whole plant cell cultures derived from explant.[7] Tissue culture offers numerous significant benefits over traditional propagation methods. The seed germination percent is low and conventional propagation method is not feasible for large scale plantation and cultivation. Large scale of production by tissue is a pre-requisite to meet the pharmaceutical needs and also for the effective conservation of plants.[8] The plant tissue culture is experimental methods of growing large number of plants the cells and tissue are obtained from plant stem,

root leaf etc. isolated under the sterile and controlled condition to encourage production of more cells in culture and to express totipotency i.e., genetic ability to produce more plants.[9] Hence, the plant cell biotechnology has evolved as new era in the field of biotechnology, focusing on the production secondary plant product. Nowadays many drugs are not available or have scarcity. So, to overcome this problem we should accept different method by which drug may be available for market demand. In such a scenario like tissue culture, artificial propagation is used as means.

In comparison to demand there is no or less cultivation of *Vacha* (*Acorus calamus Linn*.) and its unrestricted exploitation from *jungles* by industries is continuous. This is making the plant endangered and research on *Medhya dravya* as tissue culture *Vacha* (*Acorus calamus Linn*.) are knee of interest as these drugs they are capable of improving intelligence, memory according to *Ayurveda*. *Vacha* (*Acorus calamus Linn*.) is a very good memory enhancer used for improvement of intelligence memory and revitalation of sense organ. This is making the plant endangered.

The plant mentioned as *Vacha* (*Acorus calamus Linn*.) under *Ayurvedic* literature specially possess *Medhya* properties used for mental disease like epilepsy it can be help in various neurological disorders. In *Ayurveda Vacha* (*Acorus calamus Linn*.) is used in many diseases such as *Unmad*, *Apasmar* (Epilepsy), *Jwara* (fever), *Smrutiharsha*, *Vakkruti*, *Balaroga* (Paediatrics), *Shwas* (Breathing), *Kasa* (Bell stone), *Atisara* (Diarrhoea), *Arsha* (Haemorrides), *Shool*, *Krimi* (worm), *Medoroga* (Obesity), *Vatavikar* and *Shirorog.*[10] The Rhizome is considered as the useful part of the plant and it has been reported to possess tranquilizing, antimicrobial, antidiarrheal, neuroprotective, anti-inflammatory and analgesic activity. Lot of studies were carried out on phytochemical study plant of *Vacha* (*Acorus calamus Linn*.) as well as *Medhya Karma* (Memory Enhance Activity) study. In this study *Shodhit Vacha* will be use it has been mentioned in *Chakraddatta* and *Bhaishajya Rattnavali*, as per classical reference, the rhizome is to be boiled successively cow urine i.e., *Gomutra*, *Panchapallava Kwath* and *Alambusha* followed by *Bashapa Swedan*. Thus, comparative study of *Vacha* (*Acorus calamus Linn*.) collected from natural habitat and grown from different part of plants through tissue culture was not done as well the study of these two-drug analysis.

Hypothesis

Vacha (Acorus calamus Linn.) collected from Natural habitat and grown through tissue culture might have same or more amount of chemical constituents and possess *Medhya Karma* (Memory Enhance Activity) In Mice.

Research question

Is there any difference in Phytochemicals constituents and *Medhya Karma* (Memory Enhance Activity) of Naturally grown *Vacha (Acorus calamus Linn)* and *Vacha (Acorus calamus Linn.)* propagated by Tissue Culture in Mice?

Aim

To comparative study of Phytochemical and *Medhya karma* (Memory Enhance Activity) of naturally grown *Vacha (Acorus calamus Linn.)* and *Vacha (Acorus calamus Linn.)* propagated by Tissue culture in Mice.

Objective

1. To study the phytochemicals constituents of Naturally grown *Vacha (Acorus calamus Linn.)* and *Vacha (Acorus calamus Linn.)* propagated by tissue culture.
2. To Study the *Medhya Karma* (Memory Enhance activity) of naturally grown *Vacha (Acorus calamus Linn.)* and *Vacha (Acorus calamus Linn.)* propagated by tissue culture.
3. To standardize *Vacha (Acorus calamus Linn.)* collected by two methods.
4. To compare the phytochemicals constituents in *Vacha (Acorus calamus Linn.)* grown naturally and *Vacha (Acorus calmus Linn.)* propagated by tissue culture.
5. To compare the *Medhya karma* (Memory Enhance Activity) of naturally grown *Vacha (Acorus calamus Linn.)* and *Vacha (Acorus calamus Linn.)* propagated by tissue culture.

Methods: Approval from the ethical committee is received

Study design: Experimental analytical study.

Place of study: The tissue culture method of *Vacha* (*Acorus calamus Linn*.) experiment will be carried out in the Dr. Babasaheb Ambedkar University Laboratory and institutional animal house.

1. Drug study

a) Location – Dr. Babasaheb Ambedkar University Laboratory will be preferred for drug Analysis

b) Duration – 2-3 months Approx.

2. Animal study –

a) Location – Data Meghe Institute of Medical Science (Deemed to be university). Sawangi (Meghe), Wardha. Certified by Institutional Animal Laboratory will be preferred for Study Analysis

b) Duration – 2-3 months Approx.

c) Analysis

i. Elevated Plus Maze

ii. Morris Water Maze

Prior administration of trial drug the animals are allowed to get routine with the apparatus maze (EPM & MWM). With the apparatus Elevated plus Maze and Morris water maze, prior admiration of trial drug the animals are trained to move from starting quadrant to reach hidden platform in the target quadrant.

MATERIALS

1) Plant materials for study of drug

The procedure was required two different kind of plant material.

Sample A- Root of naturally grown *Vacha* (*Acorus calamus Linn*.)

Sample B- Root of *Vacha* (*Acorus calamus Linn*.) cultivated by tissue culture.

Table 1: Plant material.

Sr. No.	Groups	Particulars
1	Sample A	Specimen of *Vacha (Acorus calamus Linn.)* rhizome will be self-collected from Natural habitat
2	Sample B	Specimen of *Vacha (Acorus calamus Linn.)* rhizome will be self-cultivated by tissue culture method.

Sample size

Animal population

Total 36 Mice of either sex weighing 20-80 gm aged 8 weeks max. will be required for the present study. The animal (4-5 per cage) will be kept in laboratory 2 weeks prior to experimentation. The animal will be kept at room temp 21+_ 1.5^0c. with 60% relative humidity, and 12hour light /dark cycle.

- Mice will be divided into 6 groups consisting of 6 mice in each group.

- The selected dose of *Vacha* (*Acorus calamus Linn*.) will be low dose 200mg/kg and high dose 400mg/kg shows significant action in Mice memory.[11]

Table 2: Group of animals.

Sr. no.	Groups	Particulars
1	Group 1	Animal served as control and were received only vehicle exposure to MWM and EPM.
2	Group 2	Animal were administered with Scopolamine (0.04 mg/kg) i.p. and were treated with (Piracetam (400 mg/kg i.p)
3	Group 3	Animals were administrated with Scopolamine (0.04 mg/kg) i.p. and treated with low dose (200 mg/kg) of *Acorus calamus Linn*.
4	Group 4	Animals were administrated with Scopolamine (0.04 mg/kg) i.p. and treated with high dose (400 mg/kg) of *Acorus calamus Linn*.
5	Group 5	Animals were administrated with Scopolamine (0.04 mg/kg) i.p. and treated with low dose (200 mg/kg) of tissue culture *Acorus calamus Linn*.
6	Group 6	Animals were administrated with Scopolamine (0.04 mg/kg) i.p. and treated with high dose (400 mg/kg) of tissue culture *Acorus calamus Linn*.

Inclusion criteria

1. Genuine, healthy, uninfected sample of *Shodhit Vacha* (*Acorus calamus Linn*.) Rhizome will be collected natural grown and tissue culture method.
2. Mice of either sex weighing between 20 to 80 gm. will be included for experiment.

Exclusion criteria

1. Unhealthy, infected, immature sample of *Vacha* (*Acorus calamus Linn*.) Rhizome will be excluded in study.
2. Animal that will be overweight and not used for another experiment will be excluded from study.

Withdrawal criteria in animal

Animal with disturbed behavioural changes will be withdrawn from study and necessary measures will be undertaken.

Outcome measures

After receiving *Vacha* (*Acorus calamus Linn.)* for a time of 1 month there will be memory enhanced in 80%of animals, it will be measure heat test, colour block test.

Primary outcome: We will be observed that phytochemicals analysis of naturally grown *Vacha* (*Acorus calamus Linn*.) and tissue cultured *Vacha* (*Acorus calamus Linn*.)

Secondary outcome: We will be observed that Memory enhancing activity Of Naturally grown *Vacha* (*Acorus calamus Linn*.) and *Vacha* (*Acorus calamus Linn*.) Cultivated by tissue culture in mice.

Statistical analysis

The progression from pattern will be utilizing paired and unpaired ANOVA test for target standard by using SPSS software

Time duration of Tissue culture *Vacha* (*Acorus calamus Linn*.) Will be cultivated by tissue culture method and grown from natural habited before 6-month animal experiment.

Time duration during animal experiment: 6 months

Methods: Data collection, management and analysis

Data collection method: As per Assessment Criteria

A) Experimental condition

1. Naturally grown *Vacha* (*Acorus calamus Linn*.) plant. Naturally grown *Vacha* (*Acorus calamus Linn*.) plants will be collected as Sample A.
2. For tissue culture of *Vacha* (*Acorus calamus Linn*.) plant will be self-collected from field. Collected plant will be authenticated by taxonomist with the help of that *Vacha* (*Acorus calamus Linn*.). The plant tissue culture will be carried out in standard Dr. Babasaheb Ambedkar university tissue culture laboratory. The following media condition will be used for tissue culture of *Vacha* (*Acorus calamus Linn*.).[12]

B) Culture medium

It will be done in Standard Biotechnology laboratory Dr. Babasaheb Ambedakar University with all standard protocols with prior permission.

The MS (Murashige and Skoog media 1982) medium will be used for experiment. Because it is most commonly used in plant tissue culture experiment in laboratory. The modification will be made to this medium by supplementing with different growth regulator at different concentration depending upon the purpose of experiment.

1. All the laboratory experiment will be conducted under defined conditions. The culture will be allowed to grow at 25+- 2^0 temperature.

2. The photoperiod will be maintained at 16-hour light and 8-hour dark light intensity at 1200-1500 Lux.
3. These seedlings will be propagated in greenhouse. After maturity of *Vacha* (*Acorus calamus Linn*.) plants rhizome will be collected and considered as sample B.
4. The *Vacha* (*Acorus calamus Linn*.) plant suffering from any pest as well as diseases will not be considered.

C) After tissue culture Sample A and Sample B analysis should be in sequence.[13]

1. Pharmacogostic study

a. Panchabhutik Pariksha
b. Specific chemical tests (wherever available as per standard protocols)
c. Macroscopic examination
d. Microscopic examination

2. Physical analysis

A. Moisture content
B. Ash value
C. Specific gravity
D. P^H of 5 % w/v suspension
E. Extractive values:
F. Water soluble extractive value
G. Alcohol soluble extractive value

3. Chemical analysis

A. Qualitative analysis

a) Test for alkaloids
b) Test for glycoside
c) Test for flavonoids
d) Test for steroids and triterpenoid
e) Test for tannins

B. Quantitative analysis

1. **HPLC**- It is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.

Note: All the above phytochemical analysis will be done to study the chemical constituent present in *Vacha* (*Acorus calamus Linn*.) in comparison with both groups.

Experimental animals

Procedure to study memory enhancing activity

These two procedures will be carried out to evaluate memory enhancing activity by two methods.

1. Morris water maze[14]

The Morris water maze is widely use to study of spatial learning memory.it is comprised in a circular pool i.e., 30 cm height and 90 cm diameter and fill with water (22^0c- 26^0c) to depth is 14cm. The pool will be located dimly, record the video camera and it's required to find invisible or slightly visible platform. With various clues, the maze will be divided into 4 quadrants. Take the mice one of this quadrant let the mice will swim and search the hidden platform and recording the time period.

2. Elevated plus maze[15]

The elevated plus maze will be determine to congestive behaviour which is measure to study of spatial long-term memory. The maze will be composed in wood. It will be kept 40 cm above the floor. Two open arm (29*5) and surrounded by short 1 cm edges because to avoid the falls and two closed arms (29*5*15cm). This experiment is based on aversion of mice to space of height. The mice will prefer the closed and protected area of maze.[16]

Housing and Husbandry

The Institutional Animal Ethical Committee the following work will be carried out at Central Animal (Preclinical) Research Facility House of Datta Meghe of Medical Science (DU), Sawangi (Meghe) Wardha. It will be carried out in accordance with the ethical guidelines prescribed by C.P.C.S.E.A. Animal welfare Division, Ministry of Environment and Forests, Govt. of India.

Animal Care and Monitoring

The total animal care and use of the experiment animal. The animal will be provided slandered animal food and water. The animal will be adapted to laboratory conditions one week prior to initiation of experiments. And all experiment will be caried out according to Institutional Animal Ethical Committee guideline for care and use of experimental animal approved by committee for the control and supervision of experiment on Animal CPCSEA.

Interpretation/Scientific implications

According the *Ayurveda Vacha* (*Acorus calamus Linn*.) is having memory booster outcome but generally *Vacha* (*Acorus calamus Linn*.) is used for other condition so our probability will be 95% positive. There will be an enhancing property of intelligence in mice by consumption of tissue grown *Vacha* (*Acorus calamus Linn*.) as well as naturally grown *Vacha* (*Acorus calamus Linn*.).

We can't use in maximum number of mice there is limitation due to animal ethical committee.

Expected results

Primarily phytochemical analysis of both naturally grown *Vacha* (*Acorus calamus Linn*.) and *Vacha* (*Acorus calamus Linn*.) cultivated by tissue culture will be revelled various phytoconstituents including alkaloids, glycoside, Flavonoids, steroids, triterpenoid and tannins etc.

Effect of Both naturally grown *Vacha* (*Acorus calamus Linn*.) and *Vacha* (*Acorus calamus Linn*.) cultivated by tissue culture in Mice in Morris water maze and Elevated plus maze will be observed.

DISCUSSION

Tissue culture will be state that concept of totipotency cell. General review of plant tissue culture from starting to till date revolution of tissue culture is described, the work about plant tissue culture on protocol of *Vacha (Acorus calamus Linn.)* will be carried out and need to conserved the species.

The literature review of *Vacha (Acorus calamus Linn.)* is mentioned as under *Ayurveda* and Morden review literature. In *Vedic kalal Vacha (Acorus calamus Linn.)* is used in Medicine and also mentioned in *Brihatrayi* and *Nighantus* having *Medhya Karma (*Memory Enhance Activity) is used for mental disease like epilepsy it can help in neurological disorders.

Savitha D. Bhat, Ashok BK, BR. Shows that Anticonvulsant activity of raw and classically processed *Vacha* (*Acorus calamus Linn*.) but not any study of anticonvulsant activity of tissue culture *Vacha (Acorus calamus Linn.).*[17]

Vineet Sharma is shows that *Vacha (Acorus calamus Linn.)* in Neurological and Metabolic Disorder evidence from Ethnopharmacology, phytochemistry, pharmacology and Clinical study but there is no study of tissue culture *Vacha (Acorus calamus Linn.)* in Phatochemicals and *Medhya Karma* (Memory Enhance activity) in Mice.[18]

The reason behind the selection of this plant is *Vacha (Acorus calamus* Linn.*)* is good memory enhancer but it is endangered species. In present study protocol, the naturally grown *Vacha (Acorus calamus Linn.)* and *Vacha (Acorus calamus Linn.)* In tissue culture will be used in memory enhance activity.

CONCLUSION

In this study *Vacha* (*Acorus calamus Linn*.) will be effective in Memory enhancing Activity. Lot of studies were carried out on phytochemical study plant of *Vacha* (*Acorus calamus Linn*.) as well as *Medhya Karma* (Memory enhance activity) study, but comparative study of Naturally grown *Vacha* (*Acorus calamus Linn*.) and Tissue culture Of *Vacha* (*Acorus calamus Linn*.) still yet not conducted in any such a type of study hence, the study will be planned. to overcome this situation current study will be carried out on components of natural grown *Vacha* (*Acorus calamus Linn*.) and *Vacha* (*Acorus calamus Linn*.) Grown by the tissue culture possessing *Medhya Karma* (Memory Enhance activity) administered in Mice.

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