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Protocol

Comparative *in vitro* antimicrobial study of Panchavalkal and modified Panchavalkal in three different extract solvents: a study protocol

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ABSTRACT

Background: Charak, Sharangdhar, Kashyap, BhavaPrakasha described Panchavalkala, is a combination of five astringent barks viz., Vata (*Ficus bengaenesis* Linn.), Udumbara (*Ficus glomerata* Roxb.), Ashvattha (*Ficus religiosa* Linn.), Parisha (*Thespesia populanea Soland excorrea*) and Plaksha (*Ficus slacor Buch*-Ham.). In Ayurveda sometimes substitute plants are described to work better for a particular medical condition. Such type of alternative drug suggested by BhavaMishra and Kaiyadeva Nighantu for classical Panchavalkala is Shirish (*Albezzia lebbeck* Linn.) in place of Parisha. Such type of altered Panchavalkal is called as modified Panchavalkal. Both Panchavalkals are essentially important in acute, chronic ulcers, wound healing and reproductive health concern women's diseases like leucorrhea, irregular or heavy bleeding etc. So, these Panchavalkals at three different extract solvents i.e., water extract, alcoholic extract and hydro alcoholic extract in comparison to standard anti-microbial drugs will be studied.
Methods: Panchavalkal and modified Panchavalkal in water, alcohol and hydro alcoholic extract will be subjected to a test antimicrobial property by agar well diffusion method. Results will be assessed on the basis of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and the zone of inhibition (ZOI) of plant extracts against the microorganism that will be measured.
Conclusions: Conclusion will be drawn on the basis of results obtained about anti-microbial activity of Pachavalkal and modified Panchavalkal in three different extracts.
Trial registration: It is taken, Ref. No. MGACHRC/IEC/February-2021/193-A

Keywords: Panchavalkal, Modified Panchavalkal, Anti-microbial activity

INTRODUCTION

Panchavalkal has one of the most versatile plants having a wide spectrum of medicinal uses. It includes a group of five drugs; these are Vata (*F. benghalensis* L.), Udumbara (*F. racemosa* L.), Ashwatha (*F. religiosa* L.), Plaksha (*F. lacorBuch*. Ham.), Parisha (*T. populnea*). This Panchavalkal is described as Kshrivriksha by Chakrapani in his text 'Chakradatta' as these are latex yielding plants. They are also known as 'Twakapanchaka' which means bark of these five plants are collectively used for its combined therapeutic effect. Narahari quoted them as 'Panchavetasa'.[1] There is no direct description of Panchavalkala in Charka Samhita but this group is used at various places like for the treatment of Raktatisarjanya-gudapak and Trishna. In Sushrutasamhita also, term Panchavalkala is not seen but panchakashya is used in yonivyapadchikitsa. Dalhan interpreted Panchakashya as Vata, Udumbara, Ashwatha, Plaksha and Gardbhand. The word Panchavalkala is used frequently in Nighantu-granthas like Dhanvantrinighantu, Madanpalnighantu, Kaiyadevnighantu, BhavPrakash. In all these texts Vata, Udumbara, Ashwatha, Parish and Plaksha are accepted as Panchavalkala. These five plants are grouped together as classical Panchavalkal. In Ayurveda,substitute plants,

Pratinidhi drugs/Abhavdravya are described. (Bhava-prakash/ Yogratnakar). These are used if the plant is unavailable in that region and/or, if the substitution is likely to work better for similar therapeutic activities and also tested on patient as per prakriti or doshaprakop or hetu.[2] The substitute drug for classical Panchavalkal that have been described in texts is Shirish (*Albezzia lebeck* Linn) as per Bhavamishra and Kaiyadeva Nighantu. Such type of Panchavalkal in which Parish is replaced with Shirish is called modified Panchavalkal. As per Bhavprakash both types of Panchavalkal have same kashayrasa, sheetvirya, varnya, rukshaguna, shotahara, Sandhankara etc.

Phytopharmacologically, Ficus plants of Panchavalkal and modified Panchavalkal produces a number of natural bioactive compounds and phytochemical screening also shows glycosides, flavons, triterpenes, tiglic acid esters, phenols, tannins, leucoanthocyanins, stigmasterol, lupeol, saponin and triterpenoids, etc. Preliminary phytochemical screening of *A. lebbeck* plant from modified Panchavalkala contains alkaloids, 14-20 glycosides, 21 phenols, flavonoids, saponin, tannins, steroids and also anthraquinone. Studies also confirmed a high concentration of phytosteroids and tannins in Panchavalkal.

Toxicity studies are also established that ingredients of Panchavalkal (Ficus plants and *A. lebbeck*) do not contain toxic compounds, not hazardous to health so safe for oral administration. So, used in the gynaecological disorders like leucorrhoea, cervicitis, valvovaginitis and prophylaxis in gynaecological surgeries in the form douche, Varti, ointment and capsules. Hand wash gel of Panchavalkal can be used for antiseptic, kwath for pre-operative skin preparation in shalya discipline. The kwatha and ointment is used in cases of nonhealing ulcers as Panchavalkal has anti-inflammatory and wound healing activity.[3]

Several researchers studied on antimicrobial properties of classical Panchavalkal and shown its efficacy. For example, Rajan M. Bhatt was one of the first to demonstrate and studied in vitro antibacterial and antifungal activity of Panchavalkal in hydroalcoholic extracts against organisms commonly affecting female genital tract. Bhatt also worked on classical and modified Panchavalkal, against bacteria and fungi commonly causing leucorrhoea, in vitro studies with single extracts or different combinations were carried out and revealed that modified Panchavalkal with Shirisha had the best antibacterial and antifungal activity.[4]

Traditionally, the Panchavalkala is used in treatment in the form of a decoction. It has to be prepared fresh every day. most of the research work done on Panchavalkal is in single extracts. Actually, extract value in a solvent gives an idea about the nature of chemical constituents present in a drug and also useful to estimate specific constituents soluble in that particular solvent used for extraction. This means extractive values useful for the evolution of the drug in particular solvent. Therefore, this study is aimed to investigate efficiency and efficacy of Panchavalkal and modified Panchavalkal in three different solvent extracts i.e., water, alcoholic and hydro-alcoholic extract with special reference to antimicrobial action.

Need of the study

Panchavalkala were preclinical as well as clinical studied for its antimicrobial activity in different forms like douches in leucorrhoea, in chronic cervicitis, Varti in vulvovaginitis, antibacterial activity of Panchavalkaladi ointment, Panchavalkala as antiseptic gel, physico-chemical study of Panchavalkala gel, Panchavalkala capsule as in prophylaxis for major gynaecological surgeries, hand wash gel of Panchavalkala can be used for antseptic, kwath for preoperative skin preparation in shalya discipline, The Kwatha and ointment is used in case of non-healing ulcers were studied.[5] (Modified) Panchavalkal is an effective quorum sensing (QS) inhibitor; it is proven mechanism of action against infections with multidrug resistant bacteria. This study confirmed antimicrobial action of modified Panchavalkal with single extract. To explore ayurvedic therapies for anti-inflammatory action and to avoid Side effects, intolerance to allopathic drugs such type of comparative antimicrobial study in three different extract solvent with less side effects and more effective is required. Normally, the water and alcoholic extracts contain different bioactive compounds. In the decoction the hydrophilic compounds are concentrated where as in the hydroalcoholic extracts hydrophobic compounds are also soluble hence to observe in which extract antimicrobial activity is more effective of Panchavalkal and modified Panchavalkal has to be seen. The traditional Panchavalkal treatment is in the form of a kwatha. It has to be prepared fresh every day. This solvent extract study helps to find a new pathway in elucidating a potent antimicrobial agent for modified Panchavalkal and Panchavalkal.This study will also help to carried out comparative quantitative study of phytochemical compositionwith HPTLC/HPLC of Panchavalkala and modified Panchavalkal. In vitro studies with single solvent extracts of Panchavalkala and Modified Panchavalkal, in common only found organisms in women were carried out but in this study three different solvents of Panchavalkala and modified Panchavalkal, against bacteria and fungi will be put in effect.

Aim and objectives

The aim and objectives of the study is to study and compare anti-microbial activity of Pachavalkal and modified Panchavalkal in water extract, alcoholic extract and hydro alcoholic extract. Objectives are to study and compare phytochemical constitutes of Panchavalkal in three different extract solvents, to study and compare phyto-chemical constitutes of modified Panchavalkal in three different extract solvents, to study and compare phytochemical constitutes of Panchavalkal and modified Panchavalkalin three different extract solvents and to

evaluate anti-microbial activity of Panchavalkal and modified Panchavalkal at three different extract solvents in comparison to standard anti-microbial drugs.

METHODS

Source of data

Comprehensive review of books, journals and previous data related to Panchavalkal and modified Panchavalkal research works will be carried out computerized search of published and unpublished works related to the study will be conducted.

Type of study

The type of study was of a comparative experimental study.

Table 1: Grouping of trial drugs with standard control of antibacterial and antifungal drugs.

Group no.	Drugs	Form	Group code
1	Panchavalkal	Water extract	PW
2	Panchavalkal	Alcoholic extract	PA
3	Panchavalkal	Hydro-alcoholic extract	PH
4	Modified Panchavalkal	Water extract	MW
5	Modified Panchavalkal	Alcoholic extract	MA
6	Modified Panchavalkal	Hydro-alcoholic extract	MH
7	Amikacin	Powder	SCB (Standard control antibacterial)
8	Amphotericin B	Powder	SCF (Standard control antifungal)

Table 2: The feasible microorganisms will be used.

Microbial culture	Microbial strains
The gram +ve bacteria	*S. aureus*
The gram -ve bacteria	*E. coli*
Fungi	*Candida albicans*

Microbial strains used in the study are as follows shown in Table 2.

Methodology used

Drug collection/ authentication

Drugs will be collected from the field as per good collection practices (GCP) guidelines and will be authenticated in approved pharmacognosy lab.

Detail of drug preparation

Collection of plant material

Panchavalkal i.e., stem bark of five Ficus trees viz. Vata (*F. bengaenesi*s Linn.), Udumbara (*F. glomerata Roxb*.), Ashavatha (*F. religiosa* Linn.), Parish (*T. populanea S. correa*), Plaksha (*F. lacorBuch*-Ham.) and modified Panchavalkal i.e., stem bark of four Ficus trees viz. Vata (*F. bengalensis* Linn), Udumbar (*F. glomerata* Roxb), Ashwatha (*F. religiosa* Linn.) Parish (*T. populenea Soland* excorea.) and Shirisha (*A. lebbeck* Linn.) free from cancer, infection will be collected from field. The plant barks will be dried in sunlight till it becomes moisture free.

Method of powder preparation

Panchavalkal and modified Panchavalka content drugs in a same proportion will be mixed respectively and powder will be prepared by following classical method of choorna kalpana. Small pieces of stem barks will be dried completely and grinding of enough size.[6] The prepared stem bark choorna will be kept in air tight container separately and together to use for physicochemical, phytochemical study and antimicrobial activity. For quality assessment of churna analytical specifications will be carried out.[7]

Physicochemical study of Panchavalkala and modified Panchavalkala churna will be done. Phytochemical study of Panchavalkala and modified Panchavalkal churna will be done.

Panchavalkala and modified Panchavalkala bark churna extract will be prepared by using a cold percolation method in Water, Alcohol and Hydro-alcohol and collected in vial for antimicrobial study.

Microbial culture for the gram +ve bacteria viz. *S. aureus*, gram -ve bacteria viz. *Escherichia coli*, and fungi *Candida albicans* will be prepared. According to the clinical and laboratory standards institute (CLSI) guideline, modified Kirby Bauer disc diffusion method will be performed for antimicrobial susceptibility test for all selected microbes. antimicrobial assay of Panchavalkala and modified Panchavalkal bark churna extract in water, alcohol and hydro-alcohol will be performed by Muller Hinton agar (MHA) plates. The test organisms will inoculate in nutrient broth and will incubate for 24 hrs. at 37 ºC. Plant extracts will be prepared in dimethyl sulfoxide (DMSO). Sixwells of 6 mm will be bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well will

be filled with 50 μl extracts: positive control amikacin for bacteria and amphotericin B for fungal isolates and negative/solvent control (DMSO), respectively. It will be allowed diffusing for about 30 minutes at room temperature and after overnight incubation at 37^0 C, plates will be observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds.[8] Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the Panchavalkala and modified Panchavalkal-The MIC of the extracts will be determined according to micro broth dilution technique. In a micro titer plate twofold serial dilutions of extracts will be prepared to obtain various concentrations. The bacterial inoculums will be added to get a final concentration of 5×105 CFU/ml in each well. The positive control i.e., amikacin will be used as a standard drug. The plate will be incubated for 24 h at 37 ºC and after overnight incubation these plates will be observed for turbidity. The plates showing the minimum turbidity will be noted for MIC. The MBC is determined by first selecting plates that showed no growth during MIC determination. A loop from each tube will be subculture on to free agar plates, incubated for overnight at 37 ºC.The least concentration, at which no growth will be observed will note and considered as the MBC.

DISCUSSION

In traditional medicine of ayurveda permutation and combination of different drugs in a group are known as mishrakgana. These mishrakgana are formed based on the common useful part having similar attributes which enhance therapeutic efficacy e.g., triphala, trikatu. Panchapallava dashmula etc. Panchavalkal is one of the frequently used combination therapies described in ayurveda. Panchavalkala and modified Panchavalkal both are explained by Bhavprakash Nighantu and Kayadev Nigantu. Ficus plants from Panchavalkal are of kashay rasatmka, sheeta virya and Kapha, Pittadoshahara but Shirish drug from modified Panchavalkal is of Kashaya rasatmka, ishat ushna virya and tridoshahara though both Panchavalkala are said to be effective especially in gynecological disorders and wound healing.

In these conditions *S. aureus, E. coli, C. albicans* microbial infection is common. Traditionally, the Panchavalkala is used on these organisms in the form of a decoction and review studies also confirms the antimicrobial activity of Panchavalkal in water extract i.e.in kwath form. Individual ingredients of Panchavalkal and Panchavalkal were studied in (water) extract for antimicrobial activity and the study conclude that Panchavalkal showed more antimicrobial activity than its ingredients.[9]

Parisha (*T. populanea Soland* excorrea), content of Panchavalkal is not easily found and plant information is not much available. Therefore, it is preferable to use the modified Panchavalkal with Shirisha (*A. lebbeck* linn) in practice because of the easily availability and confirmatory antimicrobial properties of Shirish for various infections. So, needed to find potential antimicrobial action of both Panchavalkal.

Another published study showed (water) extract of (modified) Panchavalkal has an effective quorum sensing inhibitor action. Also, there is CCRS monograph study which confirmed the antimicrobial action of modified Panchavalka is more effective in hydroalcoholic extract against (classical) Panchavalkal

In present study protocol, it may conclude that anti-microbial activity of Pachavalkal and modified Panchavalkal in hydro alcoholic extract are more effective as hydrophilic and hydrobhobic bioactive compounds are concentrated than water extract and alcohol extract. So, results obtained in antimicrobial potential of different extract solvent will help in preparing most suitable modified dosage form of Panchavalkal and modified Panchavalkal which can be further developed as phytomedicine for a variety of microbial infections

CONCLUSION

Conclusion will be mentioned after the data deliberation and analysis. It may be concluded that anti-microbial activity of Pachavalkal and modified Panchavalkal in hydro alcoholic extract are more effective.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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