

Identification of Substitute for Traded Medicinal Plant *Vamshalochana* (Exudate of *Bambusa arundaniaceae* Wild.) Using Pharmacognostic, Phytochemical Parameters, Iron Estimation and *in silico* Investigation

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ABSTRACT

Background: Plants have been major component in medicine in all systems of medicine throughout the centuries. Though there are various plants used in different system of medicine, the availability of the original source has become a question due to deforestation and industrialisation. *Vamshalochana* (exudate of *Bambusa arundaniaceae* Wild.) in *Ayurveda* is used in various diseases and in various forms. It is being substituted in market with sodium silicate and ammonium silicate. This has reduced the efficacy of the formulations that are prepared out of these chemicals. **Aims and Objectives:** *Tugakshiri* (*Curcuma angustifolia*) on the other hand is used as substitute in some places instead of the plant's original exudate. Hence an analysis on the original and substituted plant phytochemical, physio chemical analysis, iron quantification and *in silico* analysis will give us a clear picture whether CA can substitute the original drug or not. **Materials and Methods:** Market samples were procured followed by Phyto and Physio chemical analysis; Iron estimation of both the plants and *in silico* analysis was completed on classically indicated two diseases COPD and Menorrhagia. **Results:** Preliminary analysis revealed that ash content was higher in BA in comparison to CA. Carbohydrate was present in both. Iron content in both was similar though statistical difference was significant ($p=0.02$). In *in silico* analysis, the binding affinity towards the diseases targets was higher in CA when compared to BA. **Conclusion:** The study concluded that *Tugakshiri* (CA) could be a suitable therapeutic substitute for the original source BA. Further evaluation on animals and human participants is required for therapeutic validation.

Keywords: Computational Pharmacology, *Curcuma angustifolia*, Exudate of *Bambusa arundaniaceae*, *In vitro* analysis, Substitute.

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INTRODUCTION

Drug is an integral part of Ayurvedic treatment. Drugs used in the manufacturing of Ayurvedic medicine are mainly derived from three sources, *Audbhida* (Plant source), *Jangama* (Animal source) and *Parthiva* (Metals and Minerals). (Agnivesha, 2009) Majority of drugs come from plant origin. But, due to non-availability of original sources, adjacent species or other species of plants have come into use in different parts of the country which are also utilized by the industries for the manufacturing of *Ayurvedic*

medicines. This practice has led to huge substitution and adulteration of original source.

The concept of drug substitution has been in practice in *Ayurveda* and is documented in classical texts of *Ayurveda* under the heading of “*abhava-pratinidhi Dravya* (substitute for unavailable drug)”, wherein an “*Abhava dravya*” (unavailable drug) is replaced by a “*pratinidhi dravya*” (substitute drug) (Shrikanthamurthy *et al.*, 1998). In *Ashtanga Hridaya* it is mentioned that, in the absence of a *dravya* in the particular *gana* (group of herbs according to different classical texts in *Ayurveda*), the other *dravyas* with similar properties can be taken in double quantities. The *Brihatrayis* (three classical litreatures of *Ayurveda*) do not mention the term *abhava-pratinidhi Dravya* (substitute for unavailable drug) (Kushwaha *et al.*, 2018). This usage occurs for the first time in *Bhavaprakasha* (16th century AD) and has been repeated in subsequent *Ayurvedic* literature (Shrikanthamurthy *et al.*, 1998).



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Here the choice of *ofabhava-pratinidhi Dravya* pairs was based on the basic principles of *Dravyaguna* (Plant pharmacology) i.e. *Rasapanchakas* (Ayurvedic Pharmacological principles). The basis of substitution may be either morphological similarities or similarity w.r.t properties with the original drug. But process of substitute identification and the reasons for using substitutes are not available in any of the classical *Ayurvedic* texts. Possibly, the unavailability of the original drugs due to geographical or seasonal changes had resulted in substitution and thus the concept came into light (Hegde *et al.*, 2011).

The species being sold in the market today and being used as substitutes are not yet proven for the particular therapeutic activity as compared to the original source. This practice is going on without any in-depth research by *Ayurvedic* practitioners and industries. Thus, for scientists in contemporary times the substitution seems unscientific and inappropriate, raising questions about the validity of their use in treatment.

Vamshalochana (*Bambusa arundinaceae* Willd. - [BA]) is a commonly used drug in many formulations like Sitopaladi churna etc., *Vamshalochana* is the exudate of bamboo (*Bambusa arundinacea* Willd. from Bombacaceae family), which is now rarely available in the market (Dr. Shastry J.L.N, 2009). *Tavakshiri/Tugakshiri/Tabasheer* (prepared from [CA] - *Curcuma angustifolia* Roxb., also called *Tikhur* and East Indian Arrowroot) is sold in Indian market, in the name of *Vamshlochan* (Kailas *et al.*, 2022). But there is a need to establish whether there is any similarity between BA and CA in terms of Pharmacognosy, phytochemical constituents and pharmacological actions in order to prove it as a substitute. Further, *in silico* study on the classically indicated disease was performed in finding out whether both the drugs have similar effect on the indicated disease and will substantiate the mode of action.

MATERIALS AND METHODS

Ethical clearance

Since the study primarily involves market procurement of plant exudates and computational analysis, a human/animal ethics approval may not be mandatory, but a statement confirming this or citing approval is required.

Procurement of the raw drug

Both the plants i.e. *Vamshalochana* (exudate of *Bambusa arundinaceae*) and *Tugakshiri* (*Curcuma angustifolia*) exudate were procured from market and authentication was done from ASU Drug Testing Laboratory. The outline of the methodology is given in Figure 1, from procurement of the samples from the market to the tests performed.

Macroscopic examination, Physiochemical and Phytochemical analysis

Ash value, Acid insoluble ash, Water soluble extract, Alcohol soluble extract, pH, sulphate ash, Water insoluble ash and Preliminary phytochemical analysis were performed on both the exudate of BA and rhizome of CA as API (Ayurvedic Pharmacopeia of India) part I, Volume 3 (GOI, API. Volume.3).

Iron estimation

The two samples, i.e. BA and CA exudates were taken 10 g each, both the samples were incinerated at 500 °C and the ash was collected. The ash was added to 2 mL of HNO₃ and 8 mL of HCl and it was filtered and the sample was made ready. The standard solution was prepared by taking Ferric chloride (FeCl₃) - anhydrous, 0.029 grams to this 100 mL of Distilled water was added. From this aliquot were prepared at the concentrations of 0.01 mg/mL (33 µL + 967 µL), 0.02 mg/mL (66 µL + 934 µL), 0.03 mg/mL (99 µL + 901 µL), 0.04 mg/mL (132 µL + 868 µL) and 0.05 mg/mL (165 µL + 835 µL). The sample solution and standard were taken and to this 200 µL of Ammonium thiocyanate was added. It was viewed under 490 nm in UV- spectrophotometer.

Statistical analysis

The obtained iron estimation values between the two plants were taken and put into GraphPad Prism and Paired 't' test was used to calculate the *p* value of the iron estimation and the significance was assessed.

Collection of Phytochemicals and Targets related to plant

Plant databases two: IMPPAT (IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics) (Mohanraj *et al.*, 2018) and Dr. Dukes (Duke, 1992) were used to collect the Phytochemicals related to both the plant along with the specified part. The phytochemicals collected were then uploaded one by one into Pub Chem (Kim *et al.*, 2025) to obtain SMILES, obtained SMILES were uploaded into ADMET Lab 3.0 for further screening of Drug Likelihood, Human Intestinal Absorption, Lipinski and Toxicity screening (Fu *et al.*, 2024). The phytochemicals were then filtered, and the SMILES of the obtained phytochemicals are entered into Swiss Target Prediction for attaining the targets related to the plant (Gfeller *et al.*, 2014).

Collection of disease target and overlapping targets

Two databases: Gene cards and HPA (Human Protein Atlas) was used to collect the targets related to disease and later the duplicates were removed (Stelzer *et al.*, 2016, Thul *et al.*, 2018). The targets related to Phytochemicals and the disease were then uploaded into Venny 2.1.0 for finding out the common targets related to both plant and the disease. (Oliver JC. Venny.)

Protein Network and Hub Genes

The common targets obtained were then uploaded into STRING database for further analysis of the interaction between the Protein by selecting the species as “*Homo sapiens*” (Szkłarczyk, *et al.*, 2023). The interaction between the targets was assessed and the network is constructed in Cytoscape 3.10.3 (Shanon *et al.*, 2003). The top 10 targets are obtained from Cytoscape, by calculating the shortest length between the targets (Chin *et al.*, 2014).

KEGG enrichment pathway

The common targets related to both the plants were then uploaded into DAVID Bio-informatics for further KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathway analysis (Sherman *et al.*, 2022). The common targets were uploaded in the list, and “Official Gene Symbol” was selected with the species as “*Homo sapiens*” the enrichment pathway analysis was done.

Topological analysis

The targets related to plant; pathways and the plant compounds are uploaded into Cytoscape 3.10.3 to perform Topological analysis (Shanon *et al.*, 2003). After this the edge-betweenness and the degree layout was calculated between the nodes. The network of the Target - Compound - Pathway was made and the merged network was created.

Molecular docking

Based on the top 10 Hub genes and the degree layout of the Phytochemicals in the topological analysis the docking between the ligand and the targets were performed. The PDB ID related to the targets were obtained from RCS PDB and the 3D structures related to the Phytochemicals are procured from Pub Chem

(Burley *et al.*, 2023). Then with Discovery Studio 2021 the water molecules were removed (Morris *et al.*, 2009). Hydrogen bond and Kollman charges are added in Auto dock tool 1.5.7 and the molecule was made ready (Morris *et al.*, 2009). With PyRx the docking between the target and ligand was done and binding affinity was calculated (Dallakyan *et al.*, 2015). The ligand with best binding affinity was visualised in Biovia 2021.

RESULTS

Authentication, Macroscopic, Physico and Phytochemical analysis

Both the samples i.e. BA and CA were authenticated and the number was given as CRF/RM/56 and 57/ 19-20. The phytochemical analyses were done for both water and ethanolic extracts. The ash value of BA 92.213% was higher than CA 0.097% and even the sulphated ash, acid insoluble ash and water insoluble ash of BA (92.985%, 76.950% and 89.705% respectively) were higher than CA. In the preliminary phytochemicals except carbohydrates, rest all the phytochemicals including the glycosides were negative. Physico-chemical and Phytochemical tests were performed and the values are tabulated in Tables 1 and 2 respectively for both the extracts.

Iron estimation

The iron estimation of the both samples was performed by UV - spectrophotometric analysis at 490 nm wavelength. The results were 0.23 mg Fe E/100 g of drug as mean concentration and with a standard deviation of 0.02 for BA while CA had a mean concentration of 0.17 mg FeE/100 g of drug with a standard deviation of 0.01, with Paired ‘*t*’ test the *p*-value was calculated and it was significant with a *p*-value of 0.02. The comparative

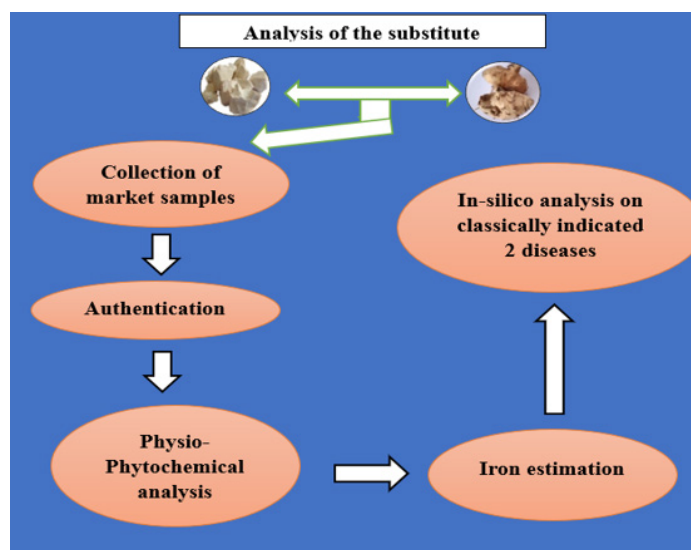


Figure 1: Outline of the methodology starting from collection of the market samples, performing *in vitro* and *in silico* analysis of both CA and BA.

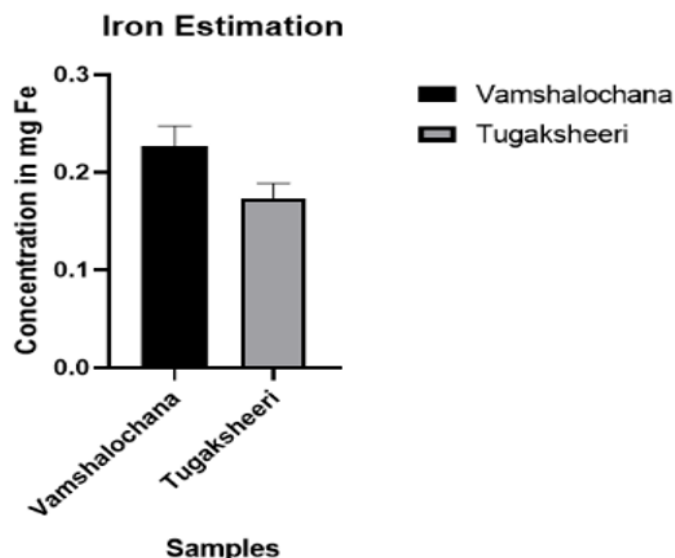


Figure 2: Iron estimation of Vamshalochana (exudate of *Bambusa arundaniaceae*) and Tugakshiri (*Curcuma angustifolia*) expressed in mg/Fe.

graphical illustration of the iron estimation between CA and BA is given in Figure 2.

Compounds and Targets related to plant

The two plants “CA” and “BA” were entered into both the databases i.e. IMPPAT and Dr. Dukes with the part specified as “Rhizome” for CA and “Shoot” and “Stem” for “BA” since the exudate part is mainly obtained from these parts. 18 Phytochemicals related to BA were obtained and 76 Phytochemicals related to CA were obtained from IMPPAT. In Dr. Dukes database, there were no phytochemicals related to both the plants. For these phytochemicals SMILES were procured from Pub Chem database. Later the Smiles obtained were uploaded and screened for Drug likeliness, Human Intestinal Absorption (HIA) and Lipinski rule. With 0.5 as cutoff, the Phytochemicals were screened for Drug likeliness and HIA in ADMET lab 3.0, along with this Lipinski rules of 5 was also taken into consideration. After this, 34 phytochemicals related CA were obtained and 5 Phytochemicals related BA were obtained. The obtained SMILES related to the phytochemicals were uploaded into Swiss target Prediction and the targets were obtained.

Disease and Common targets

The term “Menorrhagia” and “Chronic Obstructive Pulmonary Disease” were used in Gene cards and HPA databases to acquire targets related to the diseases. By selecting protein coding and with a cutoff score of 2 in Gene cards a total of 307 disease targets were obtained in relation with “Menorrhagia” and 2590 targets towards “COPD”. There were no disease targets in HPA for “Menorrhagia” while there were 16 targets were present in HPA for “COPD”. The Gene cards and HPA targets were merged and duplicates were removed and analysis of overlapping targets were done. 572 targets related to CA and 284 targets related to BA were uploaded into Venny 2.1.0 and the disease targets 307 were entered, to acquire the overlapping targets related to menorrhagia. The overlapping targets in CA and Menorrhagia are 24 in number and common targets related to BA are 18 in number. While there were 229 common targets related to CA and COPD, BA had 131 common targets with COPD.

Protein interaction and Hub genes

The common targets were uploaded into STRING database to analyze the interaction between the Genes and there was strong interaction between the targets in CA when compared to the targets in BA. While CA had strong interaction in both the common targets, BA had a reduced number of interactions between the nodes in common targets of menorrhagia but had strong interaction with common targets of COPD. The interaction network was taken to Cytoscape and the Network was constructed and the top 10 genes were calculated using CytoHubba. The top 10 Hub genes related to Menorrhagia and CA and BA were MMP2, SRC, MMP9, TNF, FLT1, MAPK1

and MAP2K1 and MMP2, MMP9, MAPK1, FGF2 and PTGS2 respectively. While the top 10 hub genes related to COPD and CA and BA are HIF1A, GAPDH, STAT3, CASP3, SRC and AKT1 and GAPDH, CASP3, BCL2, CASP8, RELA, PTGS2 and ESR1 respectively. The combined photo of CA's and BA's in menorrhagia overlapping targets, top 10 genes, Protein interaction and merged network is given in Figures 3 and 4.

KEGG pathway enrichment analysis

With DAVID Bioinformatics, the KEGG enrichment was done for the 24 and 229 common targets of CA in Menorrhagia and COPD and 18 and 131 common targets of BA in Menorrhagia and COPD. There were 114 pathways for 24 common targets of CA and Menorrhagia, while 68 pathways for the 18 common targets of BA. 172 pathways are there for 229 common targets of CA and 138 pathways are there for 131 common targets of BA. Among this top 10 KEGG pathway enrichment, Pathways in Cancer and Proteoglycans in cancer are the common pathways between the four enrichment pathways. In CA, the Pathways in

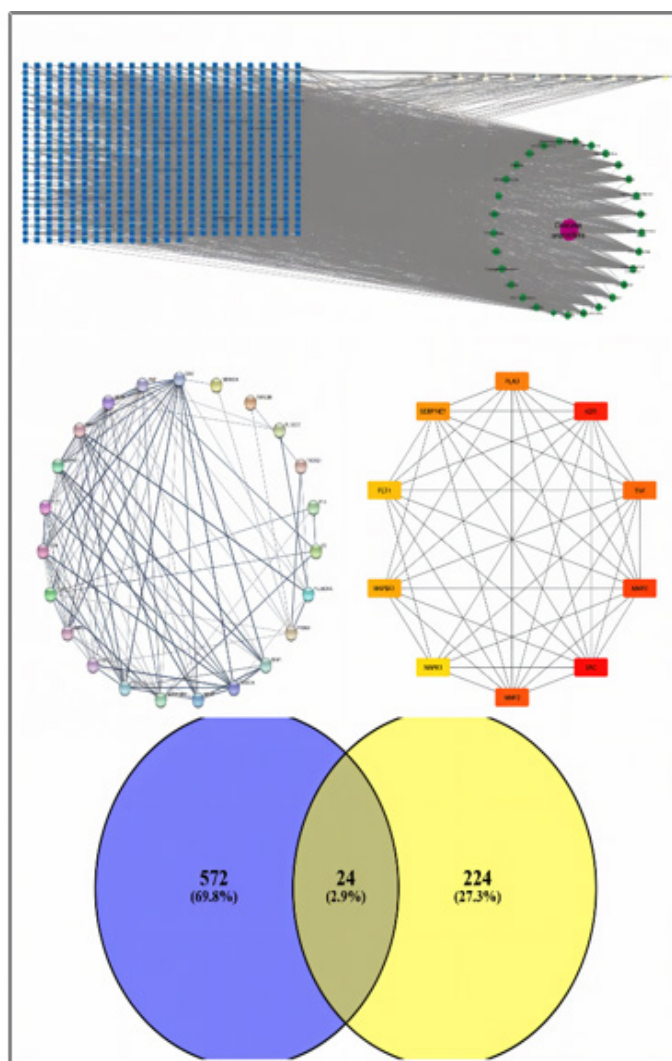


Figure 3: Overlapping targets, Hub genes, PPI and Merged network of CA.

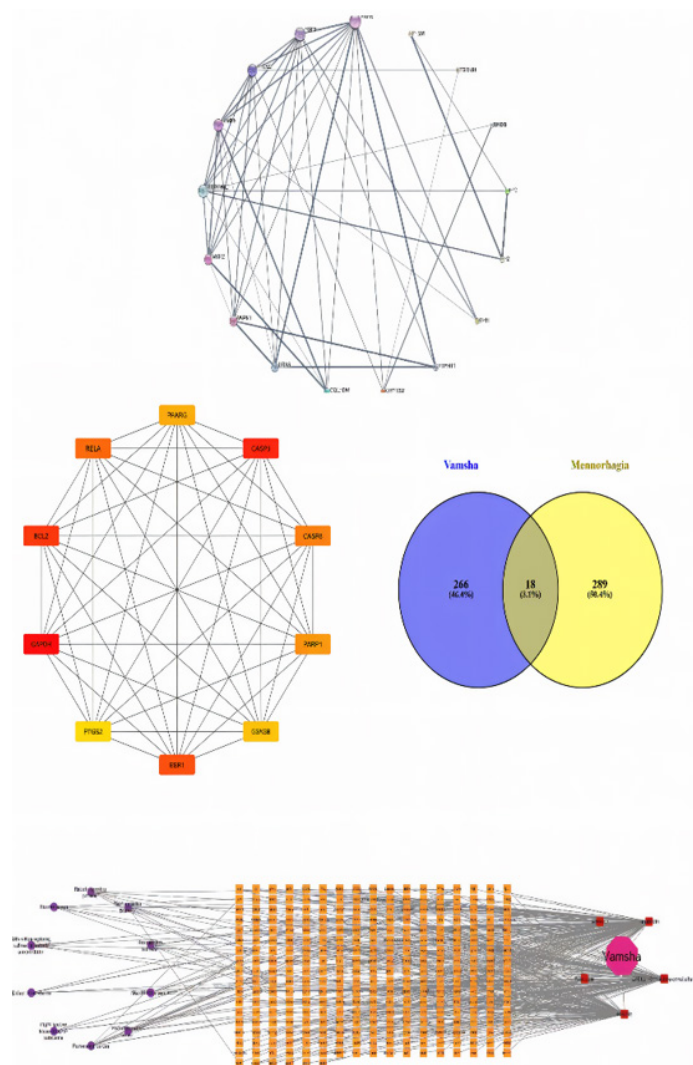


Figure 4: Overlapping targets, Hub genes, PPI and Merged network of CA.

cancer consisted of 27.9476% and 37.5% of the common targets in 24 and 229 common targets of the plant and the disease. While in BA, the Proteoglycans in cancer consisted 50% and 13.7046% of the 18 and 131 common targets of the plant and the disease.

Network construction

With 10 pathways, 24 targets and 29 Phyto-compounds in CA and 10 pathways, 18 targets and 5 phytochemicals in BA and “Menorrhagia” the topological analysis was done. In case of “COPD”, 131 targets, 5 Phytochemicals and 10 pathways related to BA and 229 targets, 29 compounds and 10 pathways related to CA were taken for Target - Compound - Pathway analysis. The degree layout for the phytochemicals were calculated. Betanine and Oxalic acid in COPD network had low degree layout with, 32 and 4 respectively while other phytochemicals had more degree layout. Eucalyptol, Beta-carophyllene and *p*-cymene has low edge count when compared to other 25 phytochemicals with a count of 84, 81 and 55 respectively. The combined photo of CA’s and BA’s

Table 1: Macroscopic and Physico-chemical analysis of exudate and root powder.

	<i>Vamshalochana</i> (exudate of <i>Bambusa arundaniaceae</i>)	<i>Tugakshiri</i> (<i>Curcuma angustifolia</i>)
Test	Values	Values
Part	Exudate	Root powder
Colour	White with blue tinge	Milky white
Taste	Slightly sour	Tasteless
Odour	Mild and characteristic	Odourless
Ash value	92.213%	0.097%
Acid Insoluble ash	76.950%	1.072%
Water soluble extract	1.200%	0.400%
Alcohol soluble extract	8.320%	0.640%
Water soluble ash	89.705%	0.00%
pH (5% solution)	10.62	6.76
Sulphated ash	92.985%	0.146%

in COPD overlapping targets, top 10 genes, Protein interaction and merged network are given in Figures 5 and 6.

Molecular docking

The molecular docking was done with Tranexamic acid, Salbutamol, Thymol, Myrtenol, Xanthorrhizol, Thymol acetate, Farnesol, ar- Turmerone, Taxiphyllin, Allantoin and Betanine with MMP9 (RCS PDB ID: 1l6j), MMP2 (RCS PDB ID: 1qib), GAPDH (RCS PDB ID: 1u8f), CASP3 (RCS PDB ID: 2h5i), BCL2 (RCS PDB ID: 4ieh), SRC (RCS PDB ID: 2src) and MAPK1 (RCS PDB ID: 5nhj). The binding affinity of the control drug i.e. Tranexamic acid was -5.6 kcal/mol for MAPK1, -6.6 kcal/mol for MMP9 and -6.6 kcal/mol for MMP2 while Betanine and ar- Turmerone had the highest binding affinity of -6.7 kcal/mol and -9.1 kcal/mol for MAPK1, -7.1 kcal/mol and -8.3 kcal/mol for MMP9 and -8.6 kcal/mol and -7.7 kcal/mol for MMP2. In COPD, the control drug Salbutamol had shown binding affinity of -5.9 kcal/mol for GAPDH, -5.3 kcal/mol for CASP3, -5.7 kcal/mol for BCL2 and -6.4 kcal/mol for SRC. Xanthorrhizol one of the phytochemicals in CA, has -6.4 kcal/mol towards GAPDH, -6 kcal/mol towards CASP3 and -7.6 kcal/mol towards SRC. Betanine one of the phytochemical present in BA showed -8.1 kcal/mol binding energy towards GAPDH, -7.4 kcal/mol towards CASP3 and -7.6 kcal/mol towards BCL2. The docking visualisation of the control drug (ligand) and the phytochemicals (ligand) towards target was visualised and the 2D and 3D visualisation images are

Table 2: Phyto-chemical analysis of exudate and root powder.

Test	Vamshalochana (<i>Bambusa arundaniaceae</i>)		Tugakshiri (<i>Curcuma angustifolia</i>)	
	Water extract	Ethanollic extract	Water extract	Ethanollic extract
Test for Carbohydrates	Positive	Positive	Positive	Positive
Test for Reducing sugar	Negative	Negative	Negative	Negative
Test for Monosaccharides	Negative	Negative	Negative	Negative
Test for Pentose Sugar	Negative	Negative	Negative	Negative
Test for Hexose Sugar	Negative	Negative	Negative	Negative
Test for Proteins	Negative	Negative	Negative	Negative
Test for Amino Acids	Negative	Negative	Negative	Negative
Test for Steroids	Negative	Negative	Negative	Negative
Test for Alkaloids	Negative	Negative	Negative	Negative
Test for Tannins	Negative	Negative	Negative	Negative
Cardiac Glycosides	Negative	Negative	Negative	Negative
Anthraquinone Glycosides	Negative	Negative	Negative	Negative
Saponin Glycosides	Negative	Negative	Negative	Negative

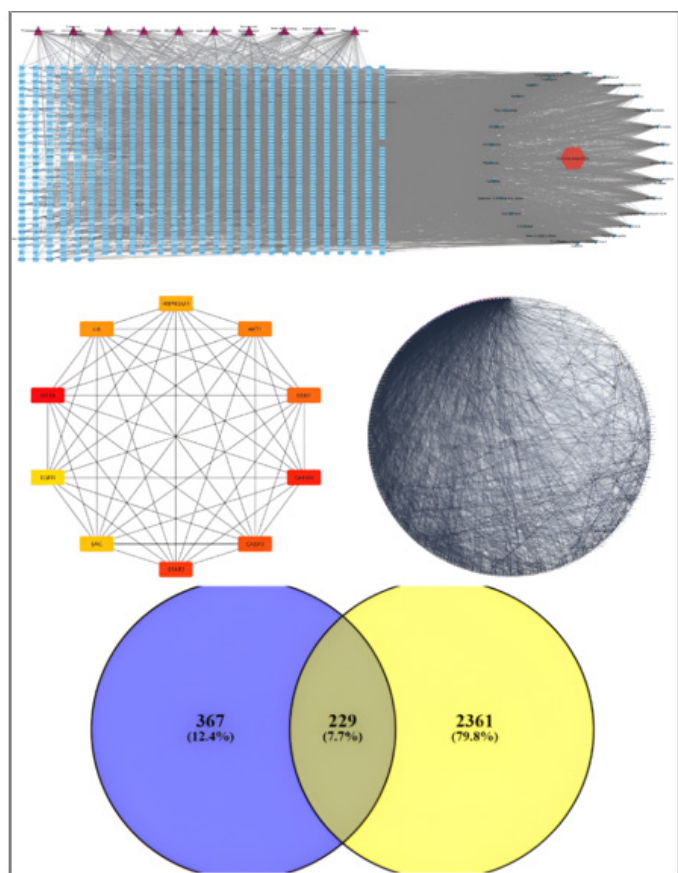


Figure 5: Overlapping targets, Hub genes, PPI and Merged network of CA.

combined and given in Figure 7, the control drug tranexamic acid and Betanine towards MAPK1 is depicted in top row of the image and the control drug Salbutamol and Xanthorrhizol towards the target SRC is portrayed in bottom row. The binding

affinity of the selected phytochemicals towards the common hub genes in Menorrhagia is given in Table 3 and the binding affinity of phytochemicals towards selected common hub genes between both the plants in COPD is given in Table 4. In Table 4, the docking of thymol, Myrtenol, Xanthorrhizol with BCL2 wasn't performed because the hub genes didn't contain the target hence the docking of these phytochemicals with the target was not done. Likewise, Taxiphyllin, Allantoin and Betanine ligand and target interaction wasn't performed because the phytochemicals didn't have the hub gene SRC hence the docking with the target wasn't done.

DISCUSSION

Substitution is a common practice in medicine since centuries. There are many drugs which are being substituted in the market but pharmacological actions of the substitutes are not established in relation to the original drug in many cases. BA is one such drug which is being substituted with CA in the market.

The Indian market currently sells artificially made BA, which could lead to ineffective medication compositions. Ammonium silicate and sodium silicate are used to create the artificial BA. These two substances are combined with water. After that, the mixture is allowed to dry. The final product of the drying process is marketed as BA. (Google books. Analysis of soil *in silica*) This kind of substitution with synthetic material might cause ayurvedic physicians and patients to lose faith in science. In such case it is necessary to establish the substitution of BA with CA with proper evidences to prove their similarity with respect to phytochemical constituents and pharmacological activities. The present study tried to find the similarity between BA and CA by analyzing and comparing their organoleptic, physico-chemical,

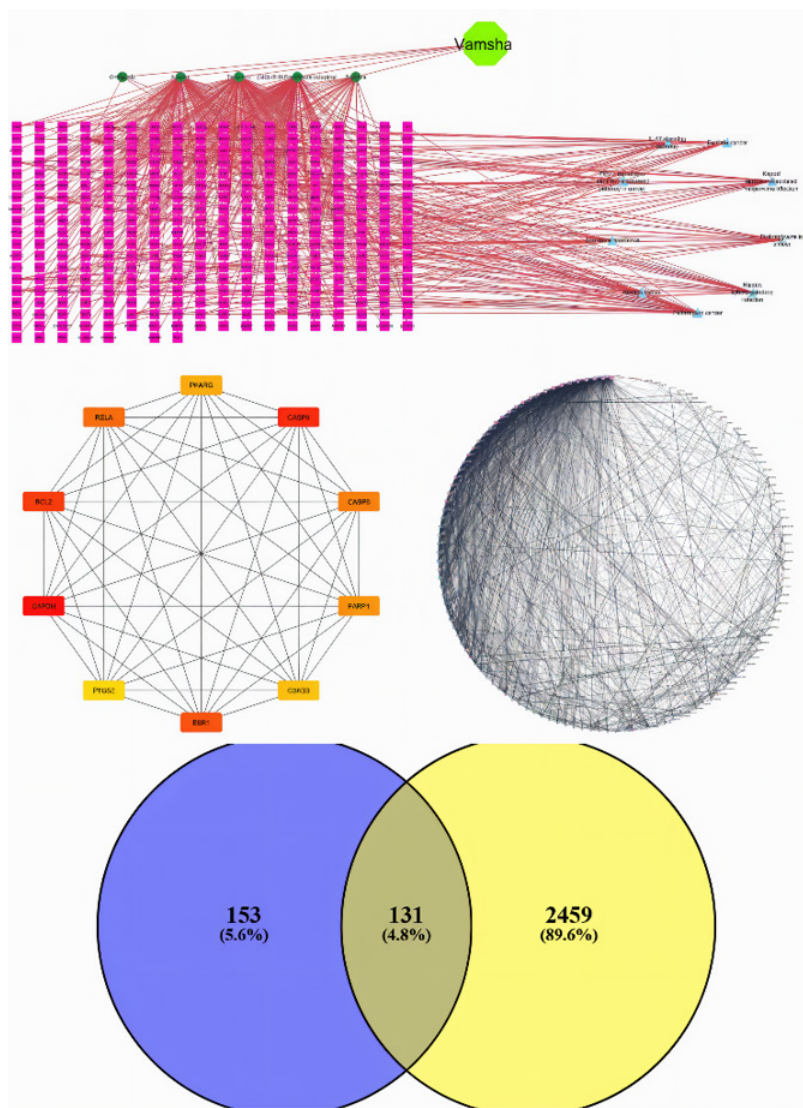


Figure 6: Overlapping targets, Hub genes, PPI and Merged network of BA.

phytochemical parameters and iron content. Further, based on the results of analytical study, two diseases - menorrhagia and COPD were chosen as per the classical indications of both the drugs and drug action was analyzed by *in silico* study.

Both the plants have similar *Rasa panchaka* (Ayurvedic Pharmacological principles) i.e. sweet taste, sweet in post digestion effect, lightness and dryness and cold in potency (Jain, 2023). Both the plants are indicated in same diseases such as *Raktapitta* (internal and external bleeding), *Kasa* (cough/ bronchitis), *Swasa* (COPD/ Bronchial asthma) and *Kshaya* (Tuberculosis) (Balakrishna *et al.*, 2023). Mainly in compound preparations as an alternate for *Vamshalochana*, the usage of *tugakshiri* is observed. CA is said to have properties such as anti-inflammatory, anticancerous, antiproliferative, hypocholesterolemic, antidiabetic, antihepatotoxic, antidiarrheal, carminative, diuretic, antirheumatic, hypotensive, antioxidant, antimicrobial, antiviral, insecticidal, larvicidal, antivenomous,

antithrombotic, anti-tyrosinase, and cyclooxygenase-1 inhibitory activities (Sharma *et al.*, 2019). BA has actions such as antibacterial, antidiabetic and anticancer properties. Hence, in regards to the *Ayurvedic* Pharmacological principles, Pharmacological action and Phytochemicals the plant CA is fulfilling the criteria of substitution. The alcohol soluble extractive value was higher in BA when compared to CA, this may be due to a greater number of secondary metabolites in BA exudate which got extracted into to the solvent. Since the preferred mode of administration of BA is not in the form of an extract, this becoming a negligible point for negating substitution.

In previous studies, the phytochemical analysis showed only presence of starch and carbohydrate was absent. Starch was present as the analysis was done exclusively on *satva* (extract). Ash values was 0.30 + 0.02 and pH was also 6.2 (Kailas *et al.*, 2022). There are slight variations in the results of present study in comparison to the previous study, this may be due to the climatic

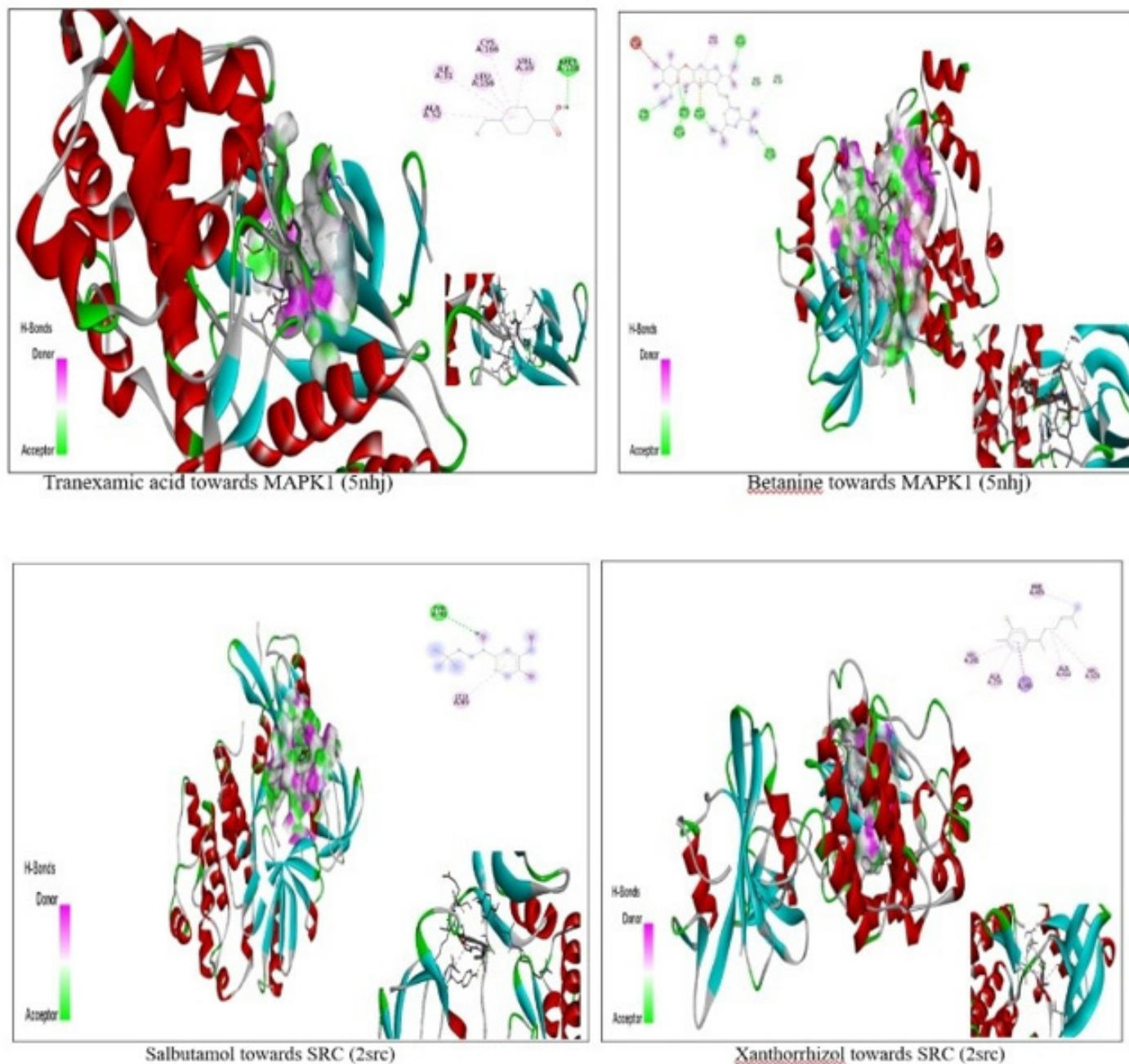


Figure 7: Visualization of control drug, Betanine towards MAPK1 and Salbutamol and Xanthorrhizol towards SRC.

change, growth factor and cultivation techniques. There was no direct reference for preliminary phytochemicals evaluation exclusively in exudates of BA, but a study on shoots of BA revealed that tannins, saponins, glycosides, alkaloids, phenols, amino acids and carbohydrates were present (Singh *et al.*, 2012).

The pH of glucose in previous studies was slightly acidic at a range of 4.8±0.2, here the presence of carbohydrate in the phytochemical analysis further justifies the acidic to neutral pH of CA (Marlerz *et al.*, 2021). But the pH of BA is basic i.e. 10.62 which may be due to the presence of inorganic compounds. The alcohol soluble extractive value was higher in BA when compared

to CA, this may be due to a greater number of secondary metabolites in BA exudate which got extracted into to the solvent. Since the preferred mode of administration of BA is not in the form of an extract, this becoming a negligible point for negating substitution.

In the present study, Iron concentration was estimated by UV spectrophotometric method. This method ensures rapid and accurate estimation of iron based on absorbance property. Ferric chloride anhydrous (FeCl₃) is a pure and stable salt of Iron which is soluble in water. It provides an accurate concentration of ferric form of iron (Fe³⁺). This method allows detection of even trace

Table 3: Binding affinity of selected phytochemicals and hub genes in Menorrhagia

Binding affinity (kcal/mol)			
Targets	MAPK1	MMP9	MMP2
PDB ID	(5nhj)	(1l6j)	(1qib)
Phytochemicals			
Tranexamic acid	-5.6	-6.6	-6.6
Thymol acetate	-6.3	-5.9	-6.6
Farnesol	-6.2	-6.1	-7.6
ar - Turmerone	-6.7	-7.1	-8.6
Taxiphyllin	-6.8	-7.4	-7.6
Allantoin	-5.9	-7.2	-6.2
Betanine	-9.1	-8.3	-7.7

Table 4: Binding affinity of selected phytochemicals and hub genes in COPD.

Binding affinity (kcal/mol)				
Targets	GAPDH	CASP3	BCL2	SRC
PDB ID	(1u8f)	(2h5i)	(4ieh)	(2src)
Phytochemicals				
Salbutamol	-5.9	-5.3	-5.7	-6.4
Thymol	-5.4	-5.3	-	-6.1
Myrtenol	-4.7	-4.8	-	-5.7
Xanthorrhizol	-6.4	-6	-	-7.5
Taxiphyllin	-6.6	-6.7	-6.3	-
Allantoin	-5.2	-5.4	-4.9	-
Betanine	-8.1	-7.4	-7.6	-

concentrations of iron. This is one of the methods used to detect iron in blood, plasma and in iron supplements in pharmaceutical industry.

The ferric iron gets converted into ferrous iron (Fe²⁺) in the intestine (duodenum) by the action of enzyme duodenal cytochrome b and later absorbed by enterocytes DMT1 (divalent metal transporter and thus becomes bioavailable (Tolkien *et al.*, 2015). Ferrous iron, though has higher absorption rates, can cause gastrointestinal side effects whereas ferric forms are better tolerated.

The iron content is required in various diseases such as Iron Deficiency Anemia (IDA), Heavy Menstrual bleeding and COPD. Iron is not just about Hemoglobin, it plays an important role in Menorrhagia, IDA and respiratory illnesses (Hardang *et al.*, 2024). Since abnormalities in iron metabolism are becoming more widely acknowledged in the pathophysiology of Chronic Obstructive Pulmonary Disease (COPD), iron plays a critical role in the condition. Dyspnoea and exercise intolerance may intensify as a result of iron deficiency or anaemia brought on by inflammation and hypoxemia in COPD. A study revealed that even in the absence of anaemia, iron deficiency is more common in COPD, especially as the disease severity increases (Munro *et al.*, 2023).

Both the plants are indicated for the same disease in *Ayurveda*, as *Pradara* (Menorrhagia) and *Swasa* (Bronchial asthma/COPD) hence both the diseases were taken into consideration for *in silico* analysis, and we found that both the plants are having common targets in both the diseases. In menorrhagia, the number of overlapping targets were less in comparison to COPD. And there were few phytochemicals though their binding affinity were higher their role in the specific disease was not analysed till now.

Menorrhagia can occur in both ovulatory (regular) and anovulatory (Irregular or absent cycles) circumstances, and both can result in severe bleeding even in the absence of structural

abnormalities. Knowing the difference between the two is crucial for management. Iron deficiency Anemia is a major consequence associated with Menorrhagia. Treatment of menorrhagia can be broadly divided into two 1. Non hormonal therapy 2. Hormonal therapy among these two, non-hormonal therapy is used and it mainly uses, Tranexamic acid, NSAID's. In hormonal therapy progestogen will be given to patients during the luteal phase of the cycle but this method of treatment is found to be less effective (Prentice, 2000).

Here Thymol acetate, ar-Turmerone, and betanine are taken for discussion as they exhibited good docking scores w.r.t Menorrhagia.

The chemical that results from adding thymol with an acetyl group is thymol acetate, which is far more stable than thymol itself. We have discussed thymol because there haven't been any prior studies on thymol acetate. Thymol produced anti-inflammatory effects in a mouse model of endometriosis by lowering inflammatory cytokine levels and immune cell infiltration in ectopic lesions (Zhang *et al.*, 2024). Menorrhagia is mostly caused by inflammation because of inflammatory mediators like prostaglandin, which can result from tissue injury and produce heavy, continuous bleeding. Another study found that thymol, at doses of 0.2 mM, suppressed uterine contractions in isolated rat uterine tissue by blocking prostaglandins, which trigger uterine contractions and ultimately result in blood loss (Bajuk *et al.*, 2022).

The sesquiterpene ar-turmerone has anti-inflammatory and anti-angiogenic effects by preventing the expression of COX-2 and iNOS in inflammatory cells and speeding up the breakdown of inflammatory mRNAs such as COX-2 and iNOS, which suggests post-transcriptional regulation (Murakami *et al.*, 2013). Angiogenesis and inflammation both play a prominent role in menorrhagia, increased prostaglandins production due to inflammation causes vasodilation and vascular fragility (Lethaby

et al., 2013) and abnormal angiogenesis causes weak endometrial vessels which leads to excess blood loss (Maybin *et al.*, 2015). Therefore, managing inflammation and angiogenesis can be strategy to treat menorrhagia.

In Human Umbilical Vein Endothelial Cells (HUVECs), TNF alpha stimulation caused endothelial dysfunction. The function of betanin (betanine) and its likely mode of action were examined using the MTT assay, western blotting, and immunofluorescence staining. Additionally in the same study, an inflammation model was created in mice using LPS to investigate the function of betanin. Betanin was discovered to increase tight junction proteins, decrease ICAM-1 and VCAM-1 expression, and prevent endothelial-mesenchymal transition by controlling autophagy. It also decreased vascular inflammation in rats. By strengthening endothelial junctions and by reducing inflammation betanin helps in menorrhagia (Li *et al.*, 2025; Bijani *et al.*, 2024).

In order to measure the levels of VEGF-A and MMP, 37 women were recruited and assessed for menstrual blood loss over the course of two cycles. According to the above-mentioned study, MMP-2 is crucial for the vascular remodeling and extracellular matrix breakdown that occurs during menstruation. Women who experience menorrhagia have lower MMP-2, which causes vessel remodeling and collapse. This results in a disruption of the vessel's structure and a malfunction in endothelial function, which may be the reason for increased menstrual blood loss (Malik *et al.*, 2006).

Chronic Obstructive Pulmonary Disease (COPD) is a progressive, treatable illness that can lead to decreased lung function, mortality if left untreated, and symptoms include restricted airflow and lung inflammation and exacerbations. To improve the condition, COPD management is crucial (Khan *et al.*, 2023).

We have considered Xanthorrhizol and Betanine for discussion as these two Phyto chemicals have shown highest docking scores among the chosen phytochemicals.

Xanthorrhizol by upregulating HO-1, NQO1, GSR antioxidant genes promotes cellular defense and provides free radical scavenging activity by which it decreases oxidative stress associated to COPD. It also reduces pro inflammatory cytokines including TNF alpha, IL-1, IL-6, IL-33 and mediators of corticosteroid resistant are blocked and functions as possible steroid re sensitizer. Xanthorrhizol lowers TH2/Th17 cytokines assists in regulation of eosinophilic and neutrophilic inflammation which is related with COPD. These effects of Xanthorrhizol suggests that it's a major phytochemical for the therapy of COPD (Liao *et al.*, 2022).

In an *in vivo* investigation, mice were given 25 and 50 mg/kg betanine for three days after intratracheal lipopolysaccharide (5 mg/kg) caused acute lung damage. *In vitro*, betanine was assessed using the MTT assay, lung histology, BALF samples, antioxidant

indicators (SOD, GSH, MDA), inflammatory mediators (iNOS, COX-2, PGE2), cytokine estimation of TNF- α , IL-1 β , and IL-6, and more. According to research, betanine has been proven to enhance cell viability, lower pro-inflammatory cytokines, and lessen lung inflammation. It also decreased the number of inflammatory cells and the production of PGE2, COX-2, and iNOS. These findings demonstrate that betanine may be a phytochemical that helps people with COPD by lowering oxidative stress and inflammation (Wu *et al.*, 2023).

The function of GAPDH is changed by post-translational modifications like succinylation, which is known to be a risk factor for both lung cancer and COPD. Airway remodeling, metabolic reprogramming, and oxidative stress are all influenced by this decreased GAPDH activity in COPD. As a result, it can be said that this disease target is crucial for managing COPD (Wang *et al.*, 2025).

In KEGG enrichment analysis, pathways of cancer were found to be similar in menorrhagia and COPD and this was also supported by literature review. Further we found more information regarding TGF- β pathway common to both the diseases.

Reduced TGF- β 1 signaling in menorrhagia patients was found to result in decreased stromal TGF- β 1 activation and decreased SMAD2/3 levels, which hinder endometrial repair and prolong bleeding (Maybin *et al.*, 2017). Another study found that TGF- β overexpression in leiomyoma-associated HMB exacerbates bleeding by inhibiting key anticoagulant factors (Sinclair *et al.*, 2011). Together we can conclude that dysfunction in TGF- β signalling plays a main role in menorrhagia.

TGF- β is increased in COPD and has a role in fibrosis, emphysema, and airway remodelling. The examination of sputum, lung tissue, and serum revealed higher levels of TGF- β , a sign of airway obstruction. TGF- β altered the recruitment and polarization of macrophages. Increased mucus secretion, angiogenesis, and Th17/Treg imbalance are other consequences of impaired TGF- β signalling. In summary, TGF- β is a key modulator of immunological dysregulation and structural lung alterations in COPD (Kraik *et al.*, 2024).

Molecular docking on the selected hub genes and target had shown us a good result and during visualization and on the binding affinity towards the control drugs Tranexamic acid and Salbutamol were comparatively lower than the phytochemicals. This has helped in both the ways, primarily the substitution of the plant CA can be done for BA. Secondly, the plant phytochemicals are equally potent enough to manage the disease, like the control drugs. In docking with Betanine towards MAPK1, there was one unfavorable covalent hydrogen implying the poor stability of the binding pose. While in Xanthorrhizol had good binding affinity of -7.5 kcal/mol towards SRC and didn't show any unfavorable bond and had good stability of the binding pose.

The physico and phytochemical analysis, iron estimation and *in silico* analysis has revealed the presence of similar qualities in both the plants making CA a valid substitute for BA. Though this study has given us a probable idea on validation of substitute the study possess some lacunas, there are no any quantification of secondary metabolites or the phytochemicals through LCMS or GCMS. The *in silico* study is solely depending on the databases which undergo regular updates on the phytochemicals and other targets. But this study has validated both ancient and modern parameters on the point of substitution.

CONCLUSION

Tugakshiri is substituted in the local markets of India for *Vamshalochana*, especially *tugakshiri* is widely available throughout India (Nath *et al.*, 2022). In *Ayurveda*, the plant exudate of *Bambusa arundinaceae* is widely recommended in classics as both single and poly-herbal preparations. So, on the physico and phytochemical analysis it revealed that rather than the inorganic content of the exudate, there were more similarities in both the plants. The iron estimation was also significant making the plant usage acceptable as a substitute.

In silico analysis of the plant in two classically indicated diseases also revealed that the mechanism of action was almost similar though there were variations in the phytochemicals. The docking gave the action of the plant phytochemicals such as Betanine and Xanthorrhizol binding affinity towards MAPK1 and SRC targets. Thus, subsequently with these data we can confirm primarily that *Tugakshiri* can be substituted for *Vamshalochana*. Further evaluation on the contents, toxicity analysis, *in vivo* assessment and clinical trials will reveal the credibility of the *Tugakshiri* as a potent substitute for *Vamshalochana*.

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ABBREVIATIONS

COPD: Chronic Obstructive Pulmonary Disease; **BA:** *Bambusa arundinaceae* Wild.; **CA:** *Curcuma angustifolia* Roxb.; **API:** Ayurvedic Pharmacopeia of India; **IMPPAT:** Indian Medicinal Plants, Phytochemistry and Therapeutics; **SMILES:** Simplified Molecular Input Line Entry System; **STRING:** Search Tool for the Retrieval of Interacting Genes/Proteins; **ADMET Lab 3.0:** Absorption, Distribution, Metabolism, Excretion and Toxicity Lab 3.0; **HPA:** Human Protein Atlas; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **PyRx:** Python Prescription, **HIA:** Human Intestinal Absorption; **DMT1:** Divalent metal transporter; **IDA:** Iron Deficiency Anemia; **HUVECs:** Human umbilical vein endothelial cells; **LPS:** Lipopolysaccharide; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

(used in the context of cell viability assay); **RCS PDB:** RCS Protein Data Bank (used with "ID"); **LCMS:** Liquid Chromatography-Mass Spectrometry; **GCMS:** Gas Chromatography-Mass Spectrometry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Divya Khare - Conceptualization, Performing *In vitro* study, performing statistical analysis, Preparing and Revising manuscript. Santhosh N - Performing *In silico* study, Preparation and Revising the manuscript. V Sri Venkata Krishnan - Conceptualization, Performing *In silico* study, Preparation and Revising the manuscript.

SUMMARY

Ayurveda classical texts have used the exudate of *Bambusa arundinaceae* (BA), both in single and compound formulations but currently due to the original drug's unavailability the exudate is prepared in an artificial manner which brings a lacuna in the treatment and the originality of the drug. On the other hand, in few places *Curcuma angustifolia* (CA) is being used. *Ayurvedic* literatures emphasizes that whenever a substitute drug is used instead of the original source the action and potency of the substituted drug shouldn't be compromised and availability of the substitute drug should be abundant. Since CA fulfills the criteria required as per classical texts, the *in vitro* and *in silico* analysis of both the plants were performed to identify whether CA can be substituted for BA. Market samples were procured and *in vitro* and *in silico* analysis were performed. Phytochemical analysis revealed the presence of carbohydrates in both the plants. Physico-chemical analysis revealed that Ash value was more in BA when compared to CA and moreover iron estimation also revealed that both the plants had similar amount of iron content though the difference was significant. *In silico* analysis on COPD and Menorrhagia was done and it showed that control drugs tranexamic acid in menorrhagia and salbutamol in COPD were showing lower binding affinity in comparison to the phytochemicals in CA and BA towards the hub genes. In conclusion, CA can be substituted for BA as it fulfills the criteria based on chemical and computational analysis. Future studies on animal and human subjects will be required to further validate this statement.

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