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Probable mode of action of *Sida Cordifolia* in Dyspareunia an In-Silico network Pharmacological approach

Research Article

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Abstract

Dyspareunia, a painful sexual disorder can be correlated to *Paripluta yonivyapath* (disorders of the genital tract) as per *Ayurveda* classics, which comes under the category of Female sexual dysfunction, the less explored clinical field in Ayurveda. *Bala*, *Sida cordifolia Linn* is the single drug that is directly indicated in this disease condition. This is the era in which the whole medical world is running behind in standardizing Evidence-based medicine (EBM) in clinical application. Network pharmacology, a branch of science that combines Network biology and polypharmacology, which predicts the mode of action of therapeutic drugs on both the interactome and the diseasome level. Here is an attempt to put forth the therapeutic efficacy of *Bala* in Dyspareunia through network pharmacology and bonding energy was demonstrated by molecular docking. Methods: Phytochemicals of *Bala* were collected from the IMPPAT database and related research articles. The disease targets were obtained from databases namely the Therapeutic target database, ChEMBL, Human protein atlas, and DisGeNet. The therapeutic efficacy of these gene targets in dyspareunia is confirmed and the conclusion is obtained from the network pharmacological ligand-target interaction methodology. The ligands and targets were retrieved from the PubChem, Protein Data Bank and docked using PyRx software. Results and Conclusion: The current study identified important Phyto-constituents like Resin Acid, Malvalic Acid, 5,7-Dihydroxy-3-Isoprenyl Flavones, Peganine, B-Phenethylamine, Ψ-(Pseudo) -Ephedrine, Coronaric Acid, Potassium Nitrate, Phenethylamine, Ephedrine, Choline which were highly modulating CYP19A1, ESR2, MAPK1, AR, MAPK3, ESR1, CYP19A1, PGR proteins related with Dyspareunia.

Key Words: *Bala,* Dyspareunia, EBM, Network pharmacology, Molecular docking.

Introduction

Life on the earth is sustained by the act of reproduction. Prevalence of Sexual dysfunction among the sexual partners prevents them from achieving Orgasm(1). *Ayurveda* classics explains that attaining *Harsha* (orgasm) is an important criterion for achieving a healthy conception(2). Dyspareunia is a sexual dysfunction in women with a global incidence of 3% and 18% (3). It can be correlated to *Paripluta (Vataja) yoni vyapath*, a condition in which females experience pain during sexual intercourse(4). A woman can suffer from Dyspareunia, either due to the lack of circulating estrogen or due to the dysregulation of the localized estrogen receptors(5). This can be treated either by increasing the level of circulating estrogen or by activating the estrogen receptors. The drugs that can activate certain pathways which can either directly or indirectly increase the estrogen bio-availability in the body can be a choice of drug in this condition. *Bala taila* is the herbal formulation which was directly indicated for local therapeutic usage in *Paripluta yonivyapth*. The mode of action of *Bala taila* can be justified as *Sthanika Vata shamaka* in Dyspareunia condition. But this work is to explore the mode of action of *Bala* in dyspareunia by enhancing the bio-availability of estrogen through an In-silico network pharmacological methodology.

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Materials and methods

Deriving the Phytochemicals:

The phytochemicals present in Sida cordifolia were derived from the IMPPAT database(6) and relevant literary works(7,8,9) The phytochemicals were analyzed with the help of Molsoft software (10) for drug-likeness properties. Then phytochemicals that satisfied Lipinski's rule of 5 (drug-likeness property) were selected and respective structural information was downloaded from PubChem software (11) and the Swiss ADME (12) tool in an excel sheet for further evaluation.

Target predictions

- Phytochemical targets: The phytochemical targets were derived from Swiss target prediction software(13).
- Disease targets: The disease targets were derived from Therapeutic Target database(14), Human protein atlas(15), and DisGeNet(16) by searching disease targets with the keyword "Female infertility"
- Pathway predictions: The pathways which can be modulated by the phytochemicals of Sida cordifolia were obtained with the help of STRING biological database(17) (via KEGG pathway analysis).
- Network construction: The network which connects the highly modulated phytoconstituents of Sida cordifolia, protein molecules, and pathways was constructed.
- Molecular docking: Three-dimensional structure of the phytochemicals were taken from the PubChem database, and the protein data of the targets were retrieved from the RCSB protein data bank(18). The Ligand-protein interaction of the molecules having lowest binding energy were visualized by using Discovery Studio visualizer(19) and PyRx(20) software.

Results

Deriving the Phytochemicals

31 phytochemicals of Sida cordifolia were obtained by fetching the available e-literature sources and IMPPAT database. All the phytochemicals were analysed in Molsoft software for the drug likeness properties and 12 phytochemicals which satisfied all the rules in Lipinski's rule were selected for further analysis. PubChem database and Swiss ADME tool were used to retrieve the structural information of the respective phytochemicals for further evaluation.

Target predictions

- Phytochemical targets: Phytochemical targets of all the 12 phytochemicals were obtained from Swiss target prediction software.
- Disease targets: The 43 disease targets were obtained from the Therapeutic Target database (4 targets), Human protein atlas (3 targets) and DisGeNet (35 targets) by searching disease target with the keyword "Female infertility"
- Matching targets: 10 phytochemicals were found to have successful targets with the disease (Table no.1)
- Pathway predictions:

  A total of 114 pathways were obtained from STRING biological database (via KEGG pathway analysis). Out of which 6 pathways were selected by considering its action on Estrogen or pathways related with Estrogen.

- Estrogen signalling pathway(21)- The activation of estrogen receptors and signal transduction are the major components of the estrogen signalling pathway's transmission.
- GnRH secretion pathway(22) - Enhances estrogen secretion
- Sphingolipid signalling pathway(23)- Sphingolipid is involved in oestrogen signalling
- Ovarian steroidogenesis(24)-Enhances oestradiol levels.
- Steroid hormone biosynthesis(25)- Enhances steroid hormones including estrogen.
- GnRH signalling pathway(22) - Enhances estrogen secretion.

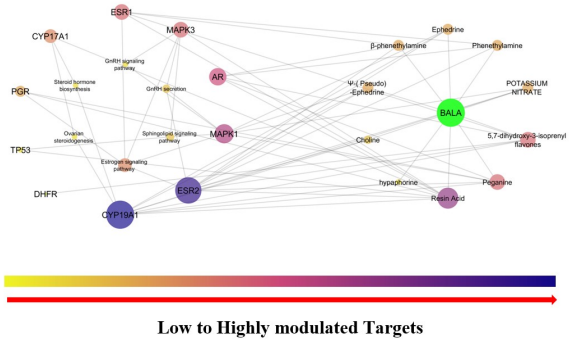
Table 1: Phytochemicals with their successful disease targets

SI. No	Phytochemicals	Name of Targets	Number Of Successful Targets
1	Ψ-(Pseudo) -Ephedrine	CYP19A1, MAPK1, ESR2	3
2	B-Phenethylamine	ESR1, ESR2, AR	3
3	(S)-(+)-Nb-Methyltryptophan Methyl Ester	CYP19A1	1
4	Hypaphorine	CYP19A1	1
5	Ephedrine	CYP19A1, MAPK1, ESR2	3
6	Choline	CYP19A1, ESR2	2
7	Peganine	CYP19A1, MAPK1, ESR2, AR, PGR	5
8	Phenethylamine	ESR1, ESR2, AR	3
9	Potassium Nitrate	CYP19A1, MAPK1, ESR2	3
10	Resin Acid	CYP19A1, MAPK3, TP53, CYP17A1, ESR1, ESR2,AR,PGR	8

- Network Construction:

The network connecting the 10 phytoconstituents of Sida cordifolia, 10 protein molecules, and 6 pathways which were highly modulated was constructed. The network was based on the edge count and size of nodes, and was interpreted using a colour and node size scale (Fig no-1).

Fig 1: Network construction of Phytochemicals of Sida cordifolia, Targets and pathways



Molecular docking:

Three-dimensional structure of the 10 phytochemicals were taken from the PubChem database, and the protein data of 5 target molecules which were having the high edge count data were retrieved from the RCSB protein data bank. The water

molecules and heteroatoms from the protein molecule were removed by using Discovery Studio visualizer and PyRx software is used to predict the binding affinity of the phytoconstituents with the respective receptors. The Ligand-protein interaction of the molecule having the lowest binding energy was visualized by using the Discovery Studio visualizer application.

- Binding affinity:

The binding affinity of the Phytochemicals and the Targets which were highly modulated are tabulated. (Table no. 2) .

Table 2: Binding affinity of the highly modulated Phytochemicals and the Targets

Sl. No	Target	Phytochemicals	Binding energy (kcal/mol)
1	AR	Resin Acid	-7.6
		Peganine	-7.1
		Phenethylamine	-5.9
		Ephedrine	-6.6
		Choline	-3.6
		Ψ-(Pseudo) -Ephedrine	-6.7
2	CYP19 A1	Choline	-3.8
		Ψ-(Pseudo) -Ephedrine	-6.2
		Ephedrine	-6.3
		Phenethylamine	-5.4
		Potassium Nitrate	-1.5
		Peganine	-6.8
		Resin Acid	-8.7
3	ESR1	Resin Acid	-9.3
		Phenethylamine	-5.5
4	ESR2	Choline	-3.8
		Ephedrine	-6.0
		Phenethylamine	-5.2
		Peganine	-7.2
		Potassium Nitrate	-1.2
		Resin Acid	-8.0
		Ψ-(Pseudo) -Ephedrine	-6.0
		Ephedrine	-6.0
5	MAPK 1	Ephedrine	-5.4
		Peganine	-6.2
		Ψ-(Pseudo) -Ephedrine	-5.4

The 3D and 2D pictorial representations of the interaction of the highly modulated receptors with phytochemicals having the lowest binding energy were represented as AR receptor with Resin acid (Fig no 2), CYP19A1 receptor with Resin acid (Fig no 3), ESR1

Fig 2: 3D and 2D representation of interaction of AR receptor with Resin acid

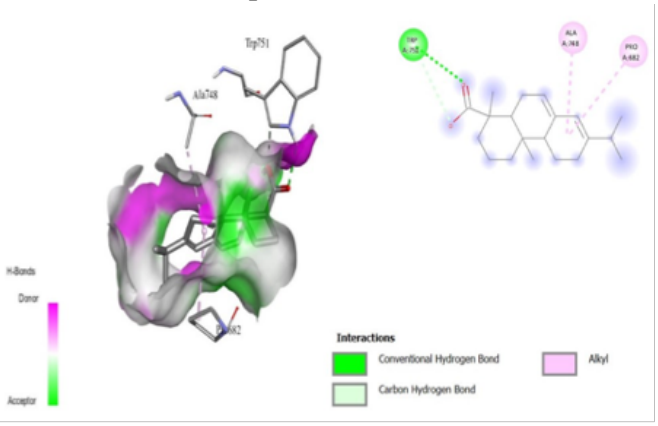


Figure 3: 3D and 2D representation of interaction of ESR1 receptor with Resin acid

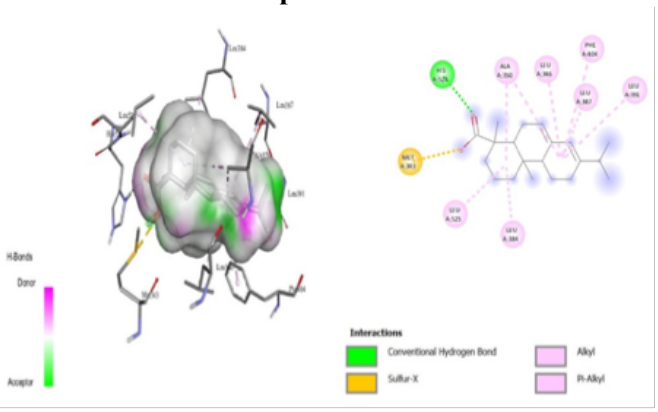


Figure 4: 3D and 2D representation of interaction of CYP19A1 receptor with Resin acid

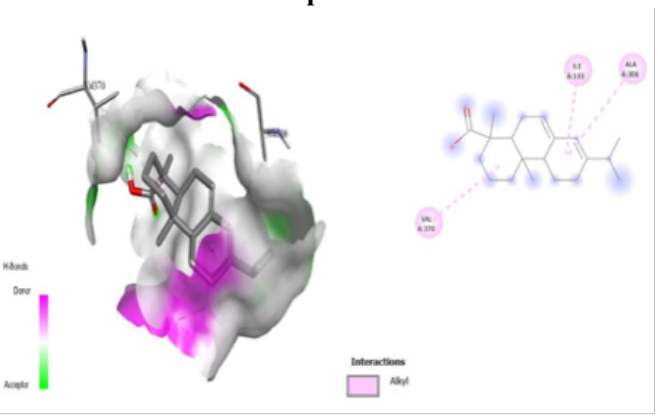
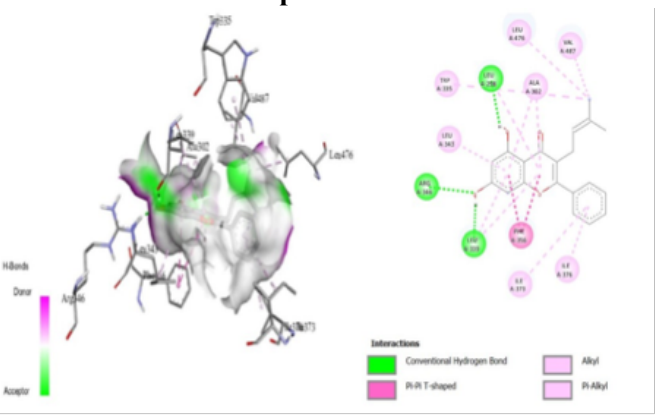


Figure 5: 3D and 2D representation of interaction of ESR2 receptor with Resin acid



receptor with Resin acid (Fig no 4), ESR2 receptor with Resin acid (Fig no 5).

The amino acids which are undergoing modulation are tabulated in Table no:3

Table no 3: List of Phytochemicals with respective receptor targets and amino acids undergoing modulation

Sl. No	Phyto-constituents	Receptor	Amino acids undergoing modulation
1	Resin acid	AR receptor	TRP A:751, ALA A:748, PRO A:682
2	Resin acid.	CYP19A1 receptor	VAL A:370, ILE A:133, ALA A:306
3	Resin acid	ESR1 receptor	MET A:343, HIS A:524, ALA A:350, LEU,A:346, LEU A:387, PHEA:404, LEU A:391, LEU A:384, LEU A:525
4	Resin acid	ESR2 receptor	ARG A:346, LEU A:343, TRP A:335, LEU A:298, LEU A:476, ALA A:302, VAL A:487, ILE A:376, ILE A:373, PHE A:356, LEU A:339

(Because Resin acid has the effective binding energy, so docking is done only for resin acid)

Discussion

Ayurveda, the wholistic science having its root in India since many millennia, have clearly explained the pathophysiology and the treatment of all the diseases. This is the era in which human population undergoes various pathological changes (like oxidative stress) in the body due to the change in their lifestyle. According to the contemporary science every new pathology is a new disease and then the whole medical world is behind the new drug discovery methodologies. But by understanding the basic concept of ayurveda, any new disease can be treated easily. The *samprapti* (pathophysiology) of any disease can be easily understood by trying to align the combination of vitiated *dosa* and *dhatus* with the *guna siddhanta* eg., Covid 19 can be correlated with *Sanipataja jwara* and line of treatment can be planned as *Samprapti vighatana (*reverse pathogenesis) or *lakshanopashama* (symptomatic). In-silico analysis predicts the probable mode of action of a medicinal drug in a more precise way. By this methodology the target of both the drug and the disease are cross matched and the identical targets are obtained and the mode of action can be explained in terms of pathways. So the phytochemicals of the medicinal drugs having antagonist action on the disease targets can be the drug of choice and its safety and efficacy can be further explored para-clinically and clinically. Many *Ekamulika dravyas* (single drug therapy) were described in our classics in many disease condition. *Bala*, was one such drug described in dyspareunia condition, which is mainly a disease connected with the estrogen deficiency or receptor dysfunction in women.

By insilico approach 10 phytochemicals of *Sida cordifolia linn* having 32 Successful targets were obtained, in that Resin acid was having the highest number of successful disease targets, that may enchance the oestrogen bio-availability by altering 6 pathways like Estrogen signalling pathway(highly modulated), GnRH secretion, Sphingolipid signalling, Ovarian steroidogenesis, Steroid hormone biosynthesis and GnRH signalling pathways and the network was constructed. Molecular docking was done for the 5 highly modulated genes (AR, CYP19A1, ESR1, ESR2, MAPK1) and the resulting binding energies were obtained. Resin acid was found to be the phytochemical with the effective binding energy and the results were tabulated (Table no 3). By this *Bala* has been proved to enhance Estrogen bioavailability and thereby can be used as a single drug therapy for Dyspareunia.

As per acharya the *bala taila yonipichu* (Vaginal tamponade) procedure was the treatment prescribed for dyspareunia. The concept of Vaginal absorption (26) has been proved in 1943 by the contemporary science that vaginal epithelium has permeability for various steroids, hormones, proteins, antigens, and other inorganic compounds. But Acharya has revealed the concept of vaginal absorption before many millennia. But clinical research has to be done on this area to understand the optimum time period required for the drug to show significant hormonal changes through vaginal absorption. *Ayurveda* is the biggest literature collection of Evidence based medicine; the only lacking area is to express the mode of action of these medicinal drugs to the experts of allied sciences in their mode of approach. More research has to be done on this area of science, for the exploration of many cost-effective modes of treatment approaches in clinical practice.

Conclusion

This article explained the mode of action of *Bala* (*Sida cordifolia*) on dyspareunia by enhancing the bio-availability of Oestrogen expressed through network pharmacological analysis and docking was done by identifying important highly modulated Phyto-constituents like Resin Acid, Peganine, B-Phenethylamine, Ψ-(Pseudo) -Ephedrine, Potassium Nitrate, Phenethylamine, Ephedrine, Choline which were highly modulating CYP19A1, ESR2, MAPK1, AR, ESR1 proteins. Clinical research work regarding this conceptualization is on progress. Female sexual dysfunction is the very less explored area of research in *Ayurveda*. More works have to be done to find out and conclude the mode of action of other drugs in this field of medical science. By exploring the multiple therapeutic uses of the available medicinal drugs, the use of particular drugs can be widely expanded in clinical practice.

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Conflicts of Interest: Nil.

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