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PHYTOCHEMICAL PROFILING AND INSILCO DOCKING STUDIES OF WRIGHTIA TINCTORIA AGAINST FOR PSORIASIS

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ABSTRACT

Wrightia tinctoria is a widely used medicinal plant. Phytochemical, antibacterial activities and antipsoriatic activities of bark of *W.tinctoria* were investigated. The Propanol extracts of *W.tinctoria* showed the presence of alkaloids, phenolics, saponins, tannins and trepenoids. The antibacterial activities of bark of *W. tinctoria* tested against *Klebsiella pneumonia, Enterobacter sp, Proteus vulgaris, Staphylococcus aureus, E coli, Pseudomonas aeruginosa*. However, many species is tested, *Enterobacter* had a maximum inhibitory effect. Whereas for antipsoriatic activity Insilco studies were performed. Phytocompounds of *W.tinctoria* has revealed a good result on the inhibition of 1PSR protein. Hence, this study suggested that *W.tinctoria* has various pharmacological activity such as anti-microbial, anti-psoriatic, anti-oxidant, etc.

KEYWORDS: Phytochemicals, *Wrightia tinctoria*, docking studies.

INTRODUCTION

Psoriasis is one of the most baffling of skin disorders. It's characterized by skin cells that multiply faster than normal. Psoriasis occurs typically on the knees and scalp. Psoriasis may also affect palms, soles of feet. Psoriasis can also associate with psoriatic arthritis, which leads to pain and swelling in the joints.[1] Types of psoriasis: Plaque psoriasis, Guttate psoriasis, Pustular psoriasis, Inverse psoriasis, Psoriatic arthritis and Erythrodermic psoriasis.Prokineticin is a secreted protein that potently contracts muscle. Prokineticins have recognized in human and other vertebrates. They involved several physiological processes like neurogenesis, angiogenesis. It may involve in cancer and regulating physiological

function that influence rhythms like hormone secretion.[2] Human Psoriasin (1psr) is s100 family consists of small acidic proteins. Psoriasin (S100A7) was initially identified as a protein highly expressed in abnormally differentiating keratinocytes derived from psoriatic skin lesions.[3] Psoriasin is an 11.7 kDa protein that highly in epidermis of patients suffering from chronic skin disease.

A number of *Wrightia tinctoria* have been extensive history in medicine system for curing several diseases. As several dosages in tribal system include plant extract in a raw basis and its efficiency was relatively same compared to that of English medicines.[4] These plant extracts were prepared and its inhibitory activity against human pathogens include Psoriasis causing organism were analyzed. This helps to study that how far it is active against a particular pathogen.[5] To, this additive work of study that the pharmacological activity of different extracts of *Wrightia tinctoria* were taken for antimicrobial activity and phytochemical analysis. And also the phytocompounds were docked with human psoriasin protein for the confirmation of psoriatic activity.

MATERIALS AND METHODS

Collection of Plant materials

The fresh barks of *W. tinctoria* were collected in the month of December [2016] from area of Vasan valley, Vaylur road Tiruchirappalli District (Tamilnadu). Plant samples were washed and shade dried at room temperature for 15 days.

Preparation of extracts and phytochemical screening

The dried plant material was powdered using a grinder. About 10 gm of powdered material was extracted in Soxhlet apparatus successively with 100 ml of each of the following solvents viz. ethanol, chloroform, propanol and methanol. The extracts obtained with each solvent were filtered through Whatman filter paper No.1and the respected solvents were evaporated (at several temperatures). The dimethyl sulphoxide (DMSO) for prior to use.

Test culture

The test bacteria used for the screening antimicrobial activity were *Klebsiella pneumonia, Enterobacter sp, Proteus vulgaris, Staphylococcus aureus E coli, Pseudomonas aeruginosa***.** Cultures were maintained as Muller Hinton agar was stored at 4ºC. From the stock culture nutrient broth and incubated at 37±1ºC for 24 hours.

Phytochemical analysis

The crude powdered plant samples were taken for the preliminary phytochemical screening to identify the presence of secondary metabolites (alkaloids, flavonoids, saponins, tannins, carbohydrates, aminoacids, steroids, glycosides, terpenoids) using standard established methods (Harbone, 1973).

Antimicrobial Activity Disc Diffusion Method

In vitro antimicrobial was carried out by disc diffusion technique in whatman no;1 filter paper disc with 4mm diameter were impregnated with known amount test sample of the disc were loaded each with 10µl of the extract by the first applying 5µl with the pipette allowed to evaporate than applying another 5µl than drying again. The positive control contained a standard antibiotic disc sterile disc use as negative control. The impregnated disc along with control (streptomycin) was kept at the center of agar plates, seeded with test bacterial cultures. The discs were then placed individually using a sterile forceps in appropriate grids which were marked on the under surface of the plates Petri plates and kept for incubation at room temperature for 24 hours. After incubation plates were observed for zones of inhibition and recorded in millimeters.

Antioxidant assay

The DPPH assay (1,1- Diphenyl, 2 –picryl hydrazyl) is basd on the reaction where the purple coloured DPPH is reducd to the yellow coloured diphenyl picrylhydrazine whn reacting with the free radicals of the sample.the free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (25-400 µg/mL). It was incubated at room temperature for forty-five minutes and the absorbance was measured at 517 nm against the corresponding blank solution. The assay was performed in triplicates. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contain DPPH and distilled water without any extract using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(\text{Acont-Atest}) \times 100}{\text{Acont}}$$

Where 'Acont' is the absorbance of the control reaction and 'Atest' is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that inhibits the formation of DPPH radicals by 50.

Molecular Docking

Target protein selection

Bioinformatics is seen as an emerging field with potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. Protein target were downloaded from database Protein Data bank (PDB). 1WWX is the PDB ID of the target protein of Human psoriasin (1psr).

Preparation of Ligand

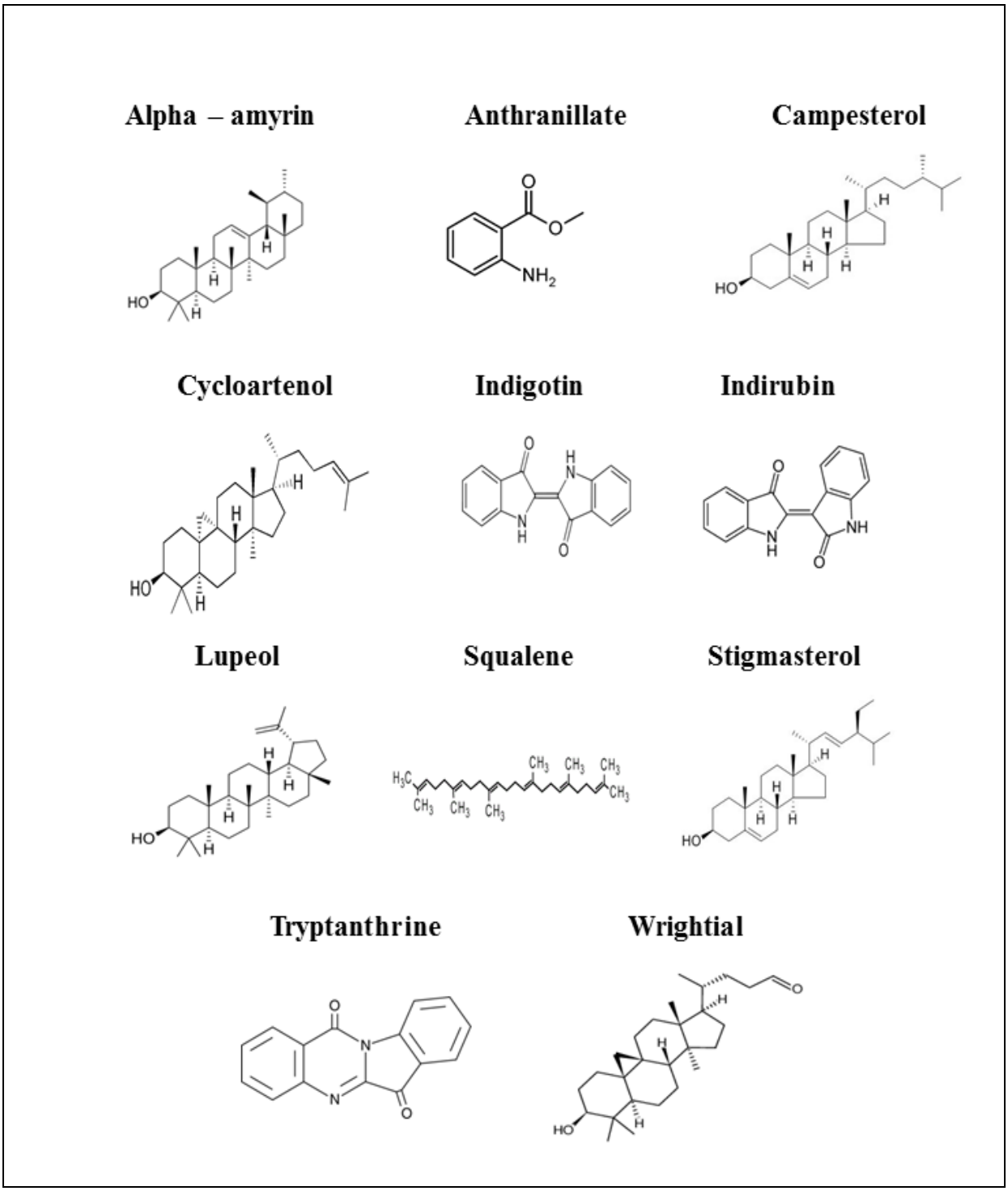


Figure 1: Chemical screened from *W.tinctoria* for the analysis of anti psoriatic activity.

UV-VIS Spectra analysis

The UV-Vis spectrum of the reaction medium at 3hours after diluting a 1ml of the sample into 4ml of distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer.

FTIR analysis

Perkin-Elmer spectrometer FTIR Spectrum in the range 4000-400 cm−1 at a resolution of 4 cm−1 was used for the analysis. The sample was mixed within KBr crystals. Thin sample disc were prepared by pressing with the disc preparing machine and placed in Fourier Transform Infrared [FTIR] for the analysis of the nanoparticles as well as for the bark powder.

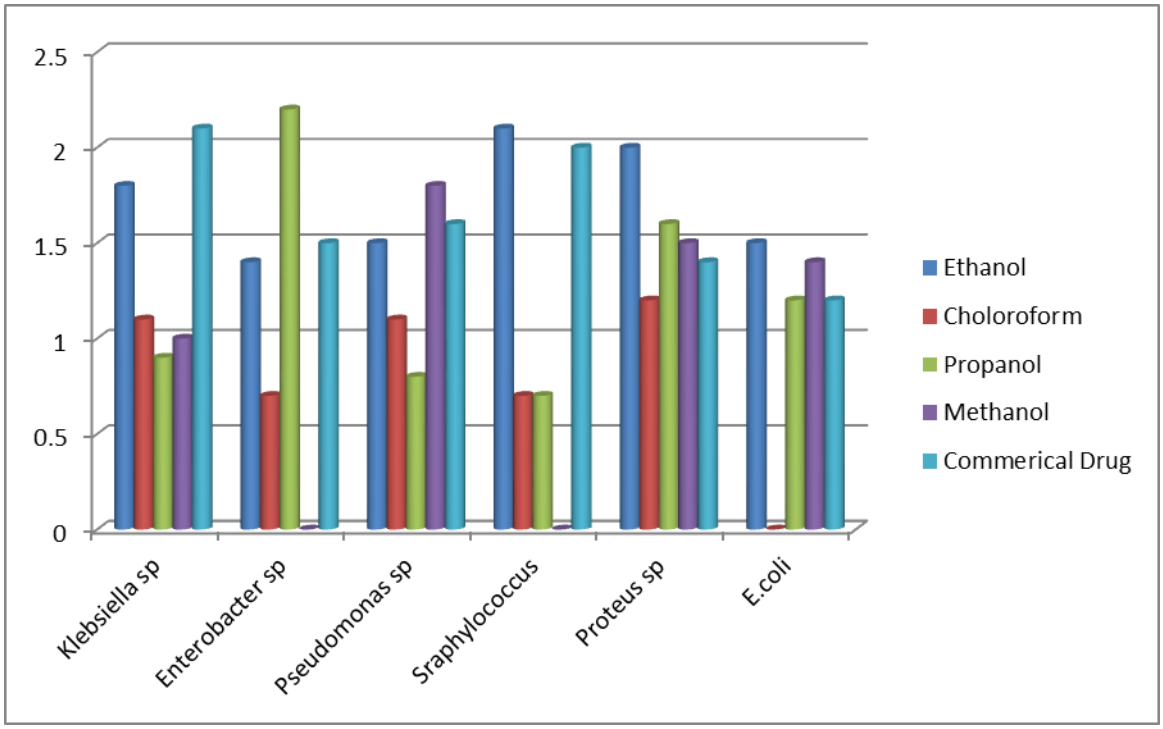
RESULT AND DISCUSSION

Antibacterial activity of propanol extract of *Wrightia tinctoria*

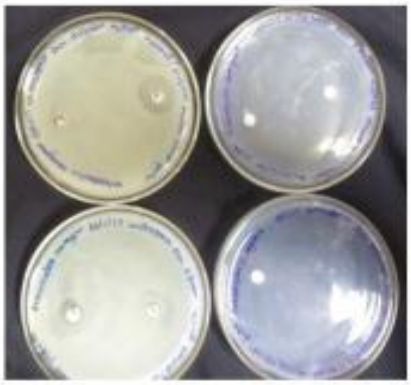
Result of the extracts with various solvents show that propanol was the more efficient solvent, followed by ethanol, methanol and chloroform. The propanol extract produced the highest yield amongst all the three extracts. This is a clear indication that the solvent system plays a significant role in the solubility of the bioactive components and influences the antibacterial activity. The high results illustrate that *W. tinctoria* showed a wide spectrum of antibacterial activity. The high potency of ethanol extract showed more potency of antibacterial effect than the other extracts. (Table.1 and Figure 1).The plants extract were compare to commercially available drugs such as (ampicillin, ciproflacin etc.). The commercial drug haves used to antibacterial activity to compare the extracts moreover the results are equal to plant extract so, we can use for commercially.

Table 1: Effects of the different extracts of the *Wrightia tinctoria* plant extract against few pathogens by agar disc diffusion method

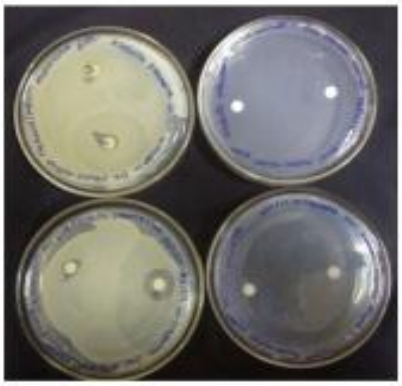
Bacterial strains	**Gram stain**	**Inhibition zone in diameters (mm / Sensitive strains)**				
Wrightia tinctoria						
A B C D E						
Klebsiella pneumonia	-	18	11	09	10	21
Enterobacter aerogenes	-	14	07	22	-	15
Pseudomonas aeruginosa	-	15	11	08	18	16
Staphylococcus aureus	+	21	07	17	-	20
Proteus vulgaris	-	20	12	16	15	14
Escherichia coli	-	15	-	12	14	12



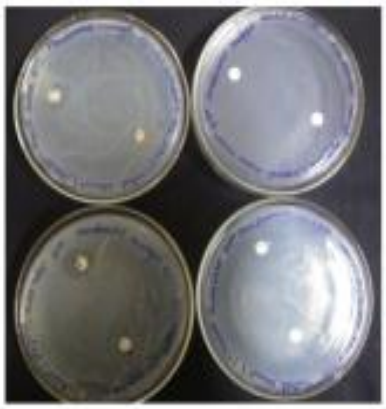
Enterobactero aerogenes



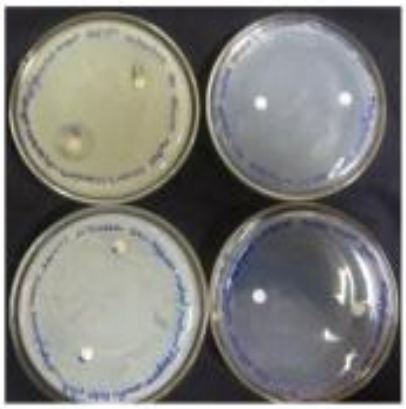
Klebsiella pneumonia

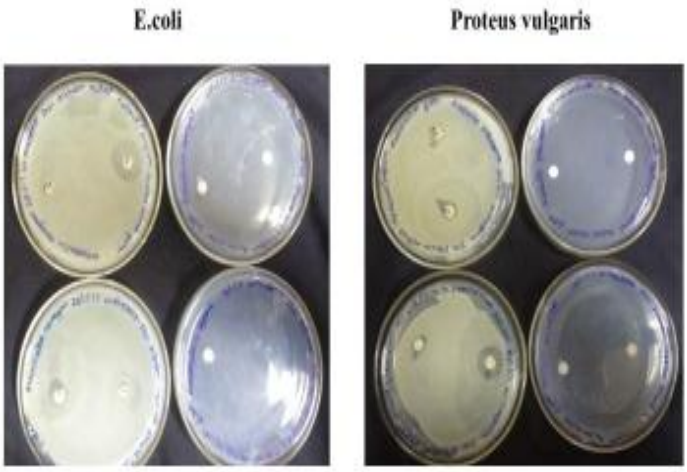


Pseudomonas aeruginosa



Staphylococcus aureus





Phytochemical analysis

The various phytochemical groups present in the chosen sample were analyzed by the standard tests and its reports the presence of alkaloids, phenoli compounds, streroids, saponins, terpenoids and tannins.

Table 2: Phytochemical Profile of *Wrightia tinctoria.*

S.NO	Phytochemicals	Wrightia tinctoria
1.	Alkaloids	+
2.	Anthraquinones	-
3.	Cardiac glycosides	-
4.	Coumarins	-
5.	Flavonoids	-
6.	Leucoanthocyanins	-
7.	Phenolic compounds	+
8.	Steroids	+
9.	Saponins	+
10.	Tannins	+
11.	Terpenoids	+
12.	Sugars	-

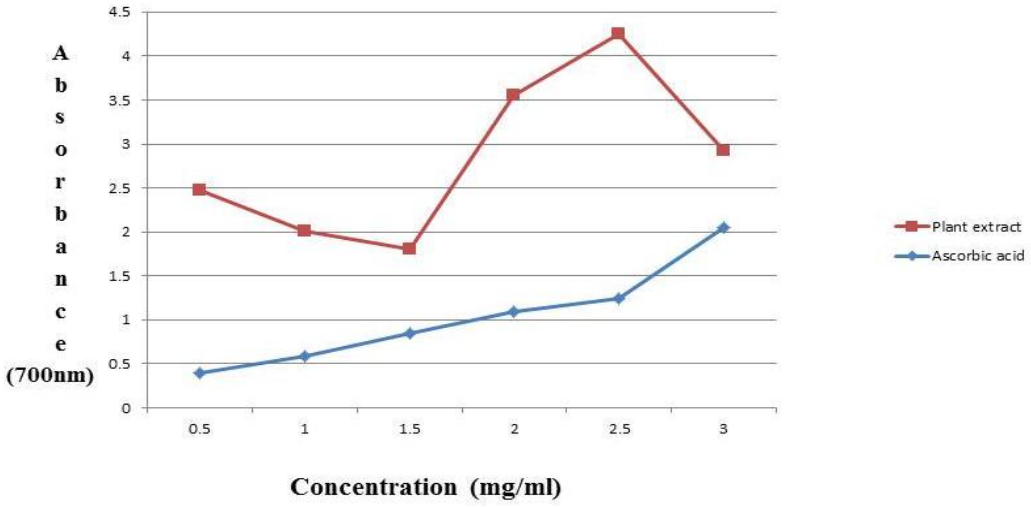
Antioxidant activity

The antioxidant activities of extracts were then evaluated by reducing power assay. The reducing capacity of a compound may serve as a significant indicator of tis potential antioxidant activity. Reducing power is the measure of the reductive ability of antioxidant and it is evaluated by the transformation Fe3+ to Fe2+min the presence of plain plant extract and encapsulated plant extracts. The reducing power of propanol extract of Wrightia tinctoria plant, summarized in Table **3**. The data showed that reducing power of propanol extracts increased with the concentration of extracts. The extracts showed potent ferric reducing

power complex that had an absorption maximum at 700nm. Antioxidant of the extract was found to be almost equivalent to one another with respect to commercial drug.

Table 3 and Figure 3: Antioxidant activity of propanol extract of *Wrightia tinctoria* and Ascorbic acid as standard.

Concentration (mg/ml)	Absorbance at 700nm (Ascorbic acid)	Absorbance at 700nm *(Wrightia tinctoria)*
0.5	0.40	2.08
1.0	0.58	1.43
1.5	0.85	0.96
2.0	1.09	2.46
2.5	1.24	3.01
3.0	2.05	0.88

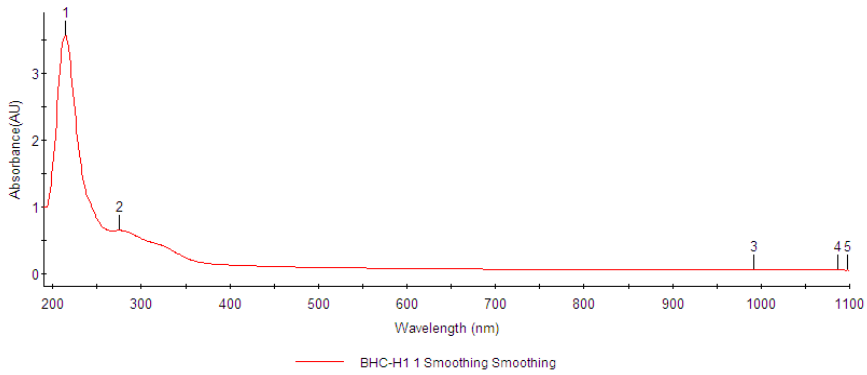


UV –Visible spectroscopy

UV-Vis spectroscopy, one of the most widely used techniques. The absorption spectrum of the plant extract solution prepared with the proposed method showed a surface plasma on absorption band with a maximum of 427 nm, indicating the presence of compound.

Table and Figure 4: Uv –Visible Spectroscopy.

S.no	Peak (nm)	Peak (Ab)
1.	214.4	3.563519944
2.	274.9	0.649787593
3.	992.0	0.065882393
4.	1,086.6	0.068072299
5.	1,097.7	0.063479021



5. FT-IR

FT-IR spectroscopy is the measurement of absorption of IR radiations by a sample plotted against the wavelength. The interpretation of the IR spectrum involves the correlation of the absorption bands (vibrational bands) with the chemical compounds in the sample. The FTIR spectrum *Wrightia tinctoria*. This represents the different functional groups of adsorbed biomolecules on the surface of plant extract.

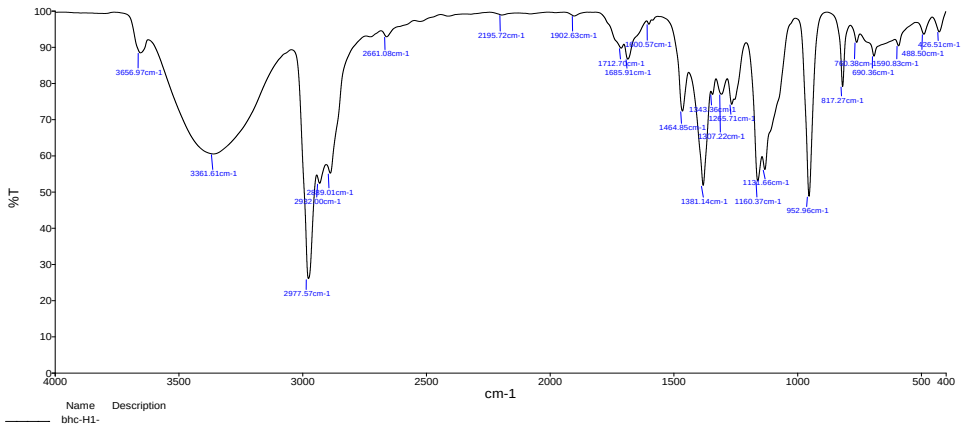


Figure 5: FTIR analysis of extract of *Wrightia tinctoria.*

In Silico Protein-Ligand Docking Studies

Human psoriasin (1PSR) activity as a major role so it is selected as a target and *Wrightia tinctoria* derivatives are tested against the target. The best compounds were screened out based on the binding energy and their interaction with the receptor molecules. For squalene, the binding results for were showing the maximum level to the active site than other tested phytocompounds. And these compounds can be used as lead for designing further treatment of Psoriasis disease.

Table 5: Binding ligands (Plant compounds).

S.no	Ligands	PubChem CID
1.	Alpha – amyrin	73170
2.	Anthranillate	227
3.	Campesterol	173183
4.	Cycloartenol	92110
5.	Indigotin	5318432
6.	Indirubin	5326739
7.	Lupeol	259846
8.	Squalene	638072
9.	Stigmasterol	5280794
10.	Tryptanthrine	73549
11.	Wrightial	72066

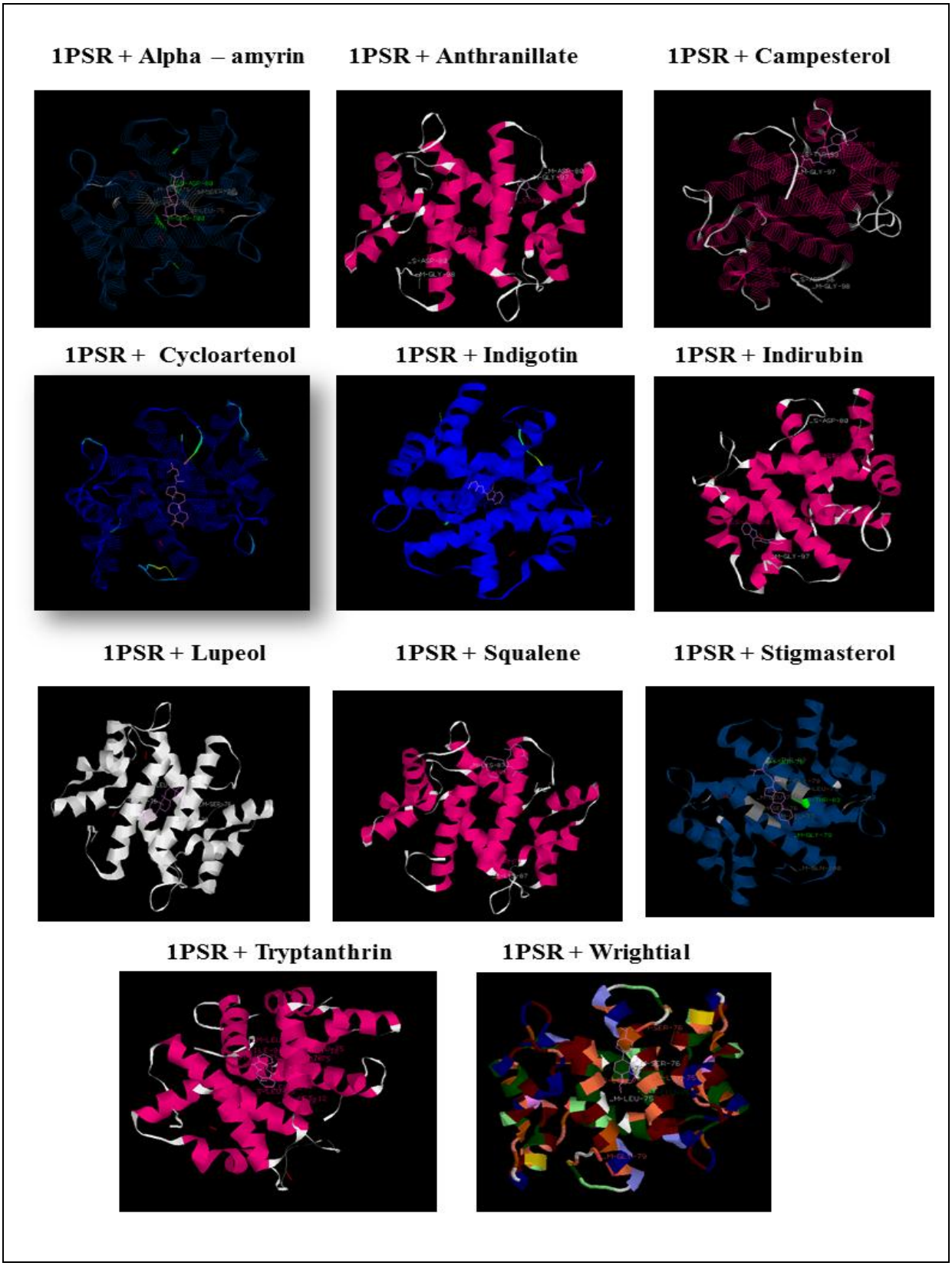


Figure 6: Binding of 1psr with different compounds of *W.tinctoria.*

Table 6: Interaction of 1psr with different phytocompounds of *w.tinctoria.*

		Compound	Energy	H-S SER 8	H-S TYR 53	H-S LYS 60	H-M GLY 79	H-S THR 83	H-S SER 99	H-S ASP 84	H-S SER 96	H-M GLY 97	V-S ILE 9	V-S MET 12	V-S TYR 53	V-S LYS 60	V-M SER 72
1	☑	1psr-Indigotin-0.pdb	-101.4	0	0	0	0	0	0	0	0	0	-3.3	-6.4	0	0	0
2	☑	1psr-stigmasterol-1.pdb	-93.5	-2.5	0	0	0	0	0	0	0	0	-1.7	-5.6	0	0	0
3	☑	1psr-Tryptanthrin-0.pdb	-92.8	0	0	0	0	0	0	0	0	0	-4.1	-6.1	0	0	0
4	☑	1psr-Cycloartenol-1.pdb	-86.7	0	0	0	0	-1.3	0	0	0	0	0	0	0	0	-3.4
5	☑	1psr-Campetosterol-1.pdb	-84.9	0	0	0	0	0	0	0	0	0	-1.1	-1.1	0	0	-1.2
6	☑	1psr-Indirubin-1.pdb	-82.3	0	-2.7	-6.9	0	0	-3.5	0	0	0	0	0	-16.5	-5	0
7	☑	1psr-alpha amyrin-0.pdb	-79.8	0	0	0	0	0	0	0	0	0	0	0	0	0	-4.1
8	☑	1psr-Wrightial-0.pdb	-78	0	0	0	-2.9	-3.4	0	0	0	0	0	0	0	0	-0.6
9	☑	1psr-anthranillate-0.pdb	-74.1	0	0	0	0	0	0	-6	-3.5	-7.8	0	0	0	0	0
10	☑	1psr-lupeol-1.pdb	-73.3	0	0	0	0	0	0	0	0	0	0	0	0	0	-6.9
11	☑	1psr-Squalene-1.pdb	-70.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0

S.NO	Compound	Energy	Vander waal's (VDW)	H-Bond	Electron
1.	1psr- alpha amyrin	-79.76	-79.76	0	0
2.	1psr- anthranillate	-74.13	-54.71	-19.42	0
3.	1psr- ampesterol	-84.86	-84.86	0	0
4.	1psr- cycloartenol	-86.74	-85.44	-1.3	0
5.	1psr- Indigotin	-101.42	-99.33	-2.09	0
6.	1psr- Indirubin	-82.34	-69.15	-13.19	0
7.	1psr- Lupeol	-73.26	-73.26	0	0
8.	1psr- Squalene	-70.61	-70.61	0	0
9.	1psr- Stigmasterol	-93.54	-89.84	-3.7	0
10.	1psr- Tryptanthrine	-92.81	-93.81	0	0
11.	1psr- Wrightial	-85.5	-85.5	0	0

CONCLUSION

The present research work concludes that *Wrightia tinctoria* is important medicinal plant with varied pharmacological properties. The plant shows the presence of many phytochemical constituents which are responsible for varied pharmacological activities. And the

phytocompounds of *W.tinctoria* can effectively inhibit the target protein. Hence the present study suggested that The *Wrightia tinctoria* can be a potent one for the treatment for psoriasis. But some evaluation must needs to be conceded out on *Wrightia tinctoria* in order to use the formulation of the plant in their practical clinical applications, which can be used for the welfare of the mankind.

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