IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 5 Ser. I (May 2022), PP 59-65
www.iosrjournals.org

Comparative study of extractive constituents from seeds of *Psoralea corylifolia*. Linn & its microbial activity.

Sheela Vasu, *Dept. of Chemistry. Smt. C.H.M College Ulhasnagar. &*
Pradnya Prabhu, *Dept. of Chemistry. K.J Somaiya college of Science & Commerce Vidyavihar.*

Abstract
Plants are used for treatment of number of diseases for thousands of years. Plants contain number of active compounds, which are responsible for various biological activities and play an important role in conventional as well as western medicine. It is also reported that phytochemicals, which are known as secondary metabolite from plants act as synergists or potentiators of antibacterial agents. This study has been done to find out the chemicals suitable for maximum extraction of the biologically active constituents as well as to find the effect of storage for one year on the chemical contents of seeds of Psoralea corylifolia Linn. There was a decrease in the total alkaloid and phenolic content of the seeds. The fresh seed extract has more alkaloid content than the seeds stored for a year. Also the maximum extraction of the constituents is found in alcoholic extractions and less in organic solvents like chloroform and pet ether. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings. Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet. Some of these by-products have been the subject of investigations and have proven to be effective sources of phenolic antioxidants.

Date of Submission: 18-05-2022 Date of Acceptance: 02-06-2022

I. Introduction

The study of natural product chemistry has principally evolved around the chemistry of bioactive phyto-components. It involves chemical characterization, isolation of bioactive components and structure determination. Understanding of this chemical diversity requires proper classification and analysis to evaluate their chemical characteristics, which is correlative with their bioactivity. Qualitative analysis of herbal extracts and major phytochemical classes like alkaloids, volatile oils, fixed oils, fats, waxes, phenyl-propanoids, flavonoids, resins, saponins, tannins, Phenols, terpenoids, glycosidal components, fluorescent substances, coloring matters, proteins, steroids & carbohydrates is therefore very necessary.

The increasing demand for herbal medicines both in the developing and developed countries inevitably leads to maintaining the quality and purity of the herbal raw materials and finished products. To ensure reproducible quality of an herbal remedy, proper control of the starting material is utmost essential. The standardization relating to herbal drugs arises from the complex composition of drugs that are used in the form of whole plant, plant parts, or extract obtained from the plants. Ensuring this quality requires knowledge and understanding of several issues like adulterations, deterioration, substitution, counterfeiting, collection, identification, and authentication of herbs. Phenolic compounds are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. When tested in edible oils, and in fish, meat and poultry products, phenolic-rich extracts have shown antioxidant activities comparable to that of synthetic antioxidants.
ADD LCMS Background & grouping of alkaloids

II. Material & Methods

The seeds purchased from authentic dealers were dried and ground to fine powder. The powder was stored in air tight container and used for the tests. Soxhlet's method was used for extraction using different solvents for the tests

Determination of soluble extractive

This method determines theamount of active constituents extracted with solvents from a given herb. Amount of herbal material extractive values are useful for determination of crude drugs and it gives an idea about the nature of chemical constituents present. The solvent used for the extraction should be in a position to dissolve appropriate quantities of decided substances

Optimization of solvent extraction

Optimization of solvent extraction as a term is used in pharmaceuticals, and involves the separation of medically active portions of a plant or animal tissues from inactive for inert components by using selective solvent in standard extraction procedure. The purpose of standardized extraction procedure for crude drugs is to obtain therapeutically desired portions and eliminate unwanted materials by treatment with the selective solvent known as menstruum.

In the present work, different solvent from polar to nonpolar were used to optimize the extractive values of *Psoralea corylifolia* seeds.

Tests

Determination of total alkaloid

200 ml of 10 % acetic acid was added in 25 grams of sample powder in 500 ml beaker and covered with aluminum foil. It was allowed to stand for 4 hours. After 4 hours. The mixture was filtered and the filtrate was concentrated on water bath to one quarter of the original volume. After the boiling was complete the collected precipitate was washed with dilute ammonium hydroxide. The residue was collected dried and weighed. This Residue is total alkaloid content present in the seeds.

Determination of total phenolic content

Folin - Ciocalteau test & HPLC analysis method were chosen to measure the total phenolic content of extract. This test was performed by referring the method developed by ***Velioglu et. all*** with some modification. The crude sample was prepared by liquefying 10 mg of extract in 10 ml of HPLC grade methanol to yield concentration of 1 mg per ml. It was diluted 10 times with deionized water in test tube. The liquid mixture was allowed to stand for 5 minutes at room temperature. About 7.5 ml of sodium carbonate was added and the test tube was shaken gently to mix them, After 90 minutes. The absorbance of the mixture was measured using UV spectrophotometer at 650, and 750 nm. A calibration curve of standard reference was established using Gallic acid range of concentration from 0.01 to 0.05mg/ ml as standard. The total phenolic content was revealed as Gallic acid equivalent in mg per 100 G of extract

Liquid chromatography with Mass spectroscopy

Instrument: Waters LCMS with QDA Detector.
Column used C 18.
Size of the columnd 50 mm X 4.6 mm ID,
particle size 3 micrometer.
Mobile Phase: Acetonitrile + Water MS grade with 0.05 % Formic acid,
Gradient Mass range: 100 to 1000 Da with + ve mode ESI source ionization.

HPLC

Instrument: Waters LC with UV Detector.
Column used C 18.
Size of the columnd 50 mm X 4.6 mm ID,
particle size 3 micrometer.
Mobile Phase: Acetonitrile + Water MS grade with 0.05 % Formic acid,
Gradient Mass range: 100 to 1000 Da with + ve mode ESI source ionization.

Microbial Ativity

Media used	Muller hilton agar for bacteria sabourauds dextrose agar for fungi
innoculum size	0.2ml
sample placed in 8 mm bore well	100 micro-liters
Incubation conditon for Bacteria	16 to 18 hours at 35 +/- 2 0 c
Incubation conditon for Fungi	24 to 48 hours at 25 +/- 2 0 c

III. Results & Calculations

Psoralea corylifolia old seeds

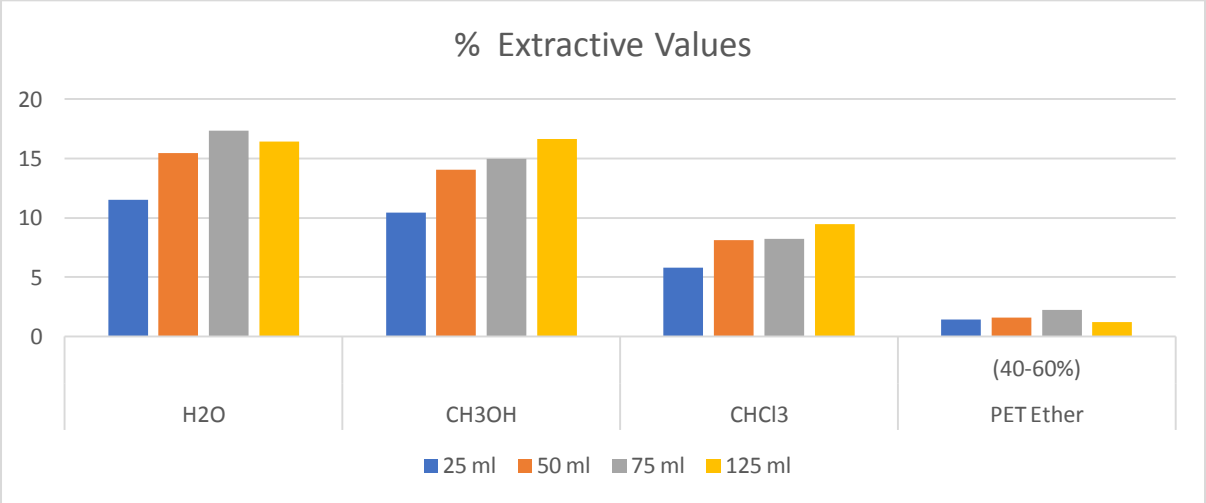
Total alcoholic extractive

Obs no	Weight in gm	% of total extractive	Mean (%) SD
1	0.901	18.001	
2	0.932	18.632	18.3913
3	0.928	18.541	

Water extractive

Obs no	Weight of ash in gm	% of total ash	Mean (%) SD
1	0.413	8255	
2	0.391	7.815	8.5813
3	0.484	9.674	

Mean % extraction for different volume of solvent				
Volume of solvent	H_2O	CH_3OH	$CHCl_3$	PET Ether (40-60%)
25 ml	11.538	10.423	5.788	1.422
50 ml	15.434	14.039	8.133	1.597
75 ml	17.332	14.987	8.233	2.246
125 ml	16.433	16.658	9.481	1.248



Total alkaloid content 125.25 mg / gm

Psoralea corylifolia new seeds

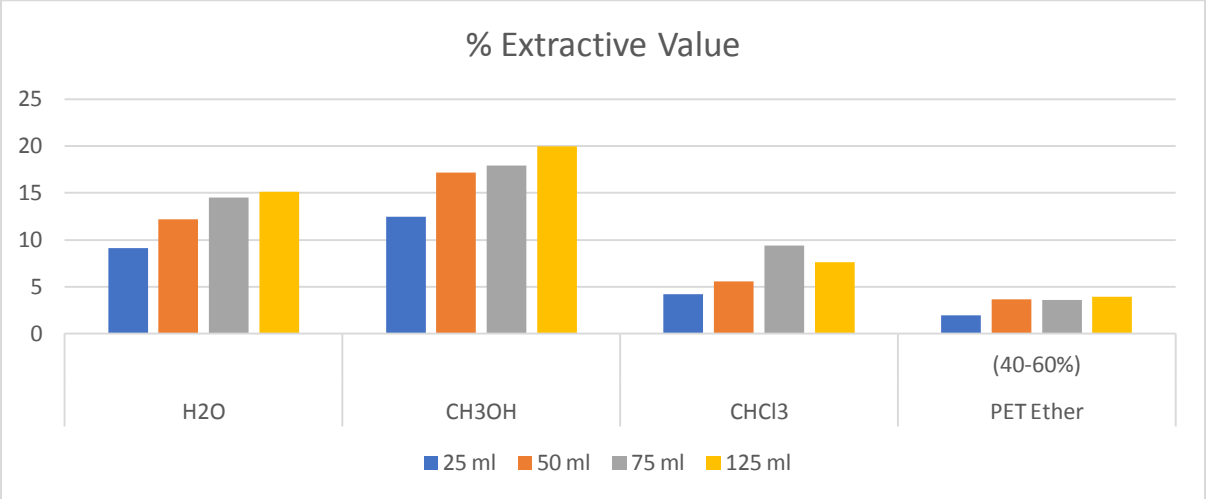
Total alcoholic extractive

Obs no	Weight of ash in gm	% of total ash	Mean (%) SD
1	0.391	19.2522	
2	0.392	19.5921	20.0452
3	0.426	21.294	

Water extractive

Obs no	Weight of ash in gm	% of total ash	Mean (%) SD
1	0.217	7.2212	7.7285
2	0.234	7.7870	
3	0.246	8.1863	

Mean % extraction for different volume of solvent				
Volume of solvent	H_2O	CH_3OH	$CHCl_3$	PET Ether (40-60%)
25 ml	9.102	12.437	4.191	1.945
50 ml	12.169	17.182	5.538	3.644
75 ml	14.513	17.932	9.366	3.594
125 ml	15.112	19.980	7.634	3.894



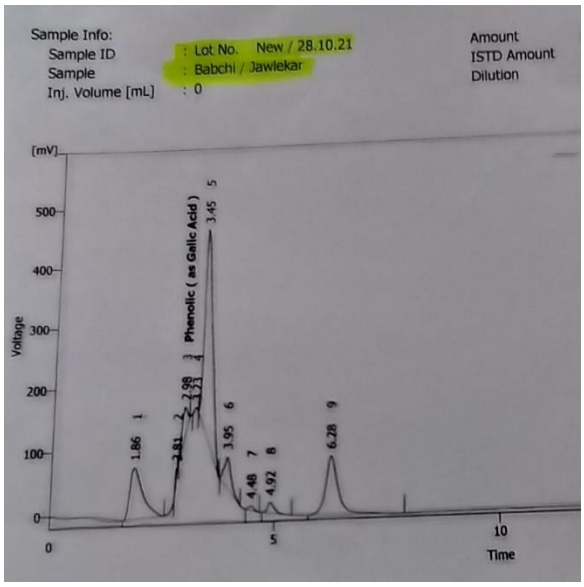
Total alkaloid content 147.0 mg / gm

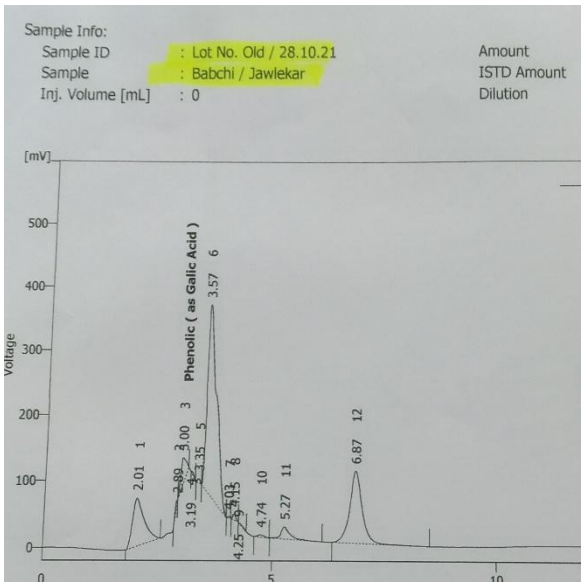
Psoralea corylifolia old		
Psoralea corylifolia new		
Micro organism	observation	inference
Escherichia coli	no zone	no effect
Pseudomonas aeruginosa	20 mm inhibiton zone	effective
Staphylococcus aureus	40 mm inhibiton zone	effective
Bacillus subtilis	31 mm inhibiton zone	effective
Candida albicans	16 mm inhibiton zone	effective
Micro organism	observation	inference
Escherichia coli	no zone	no effect
Pseudomonas aeruginosa	27 mm inhibiton zone	effective
Staphylococcus aureus	29 mm inhibiton zone	effective
Bacillus subtilis	24 mm inhibiton zone	effective
Candida albicans	25 mm inhibiton zone	effective

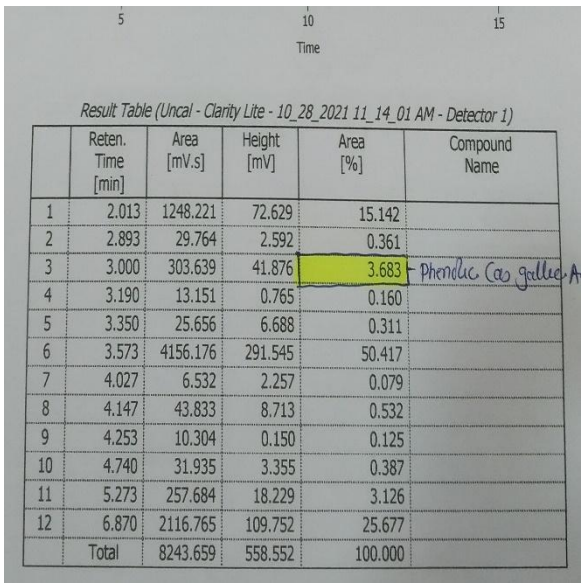
Total Phenolics

old Seeds				
	650.0 nm	750.0 nm	abs(eff)	
ethanol blank	0.0045	0.0044	0.0001	
reagent blk	0.027	0.0378	0.0108	
G 0.2 - 1ml	0.2013	0.1629	0.0384	
G 0.4 - 1ml	0.2928	0.2219	0.0709	
G 0.6 - 1ml	0.3448	0.2524	0.0924	
G 0.8 - 1ml	0.366	0.2699	0.0987	
Sample	0.5825	0.5561	0.0264	

New Seeds				
	650.0 nm	750.0 nm	abs(eff)	
ethanol blank	-0.0047	-0.0021	0.0026	
reagent blk	0.0221	0.0305	0.0084	
G 0.2 - 1ml	0.1775	0.1457	0.0318	
G 0.4 - 1ml	0.2595	0.1984	0.0611	
G 0.6 - 1ml	0.3118	0.2326	0.0793	
G 0.8 - 1ml	0.3306	0.2433	0.0873	
Sample	0.4784	0.4682	0.0102	



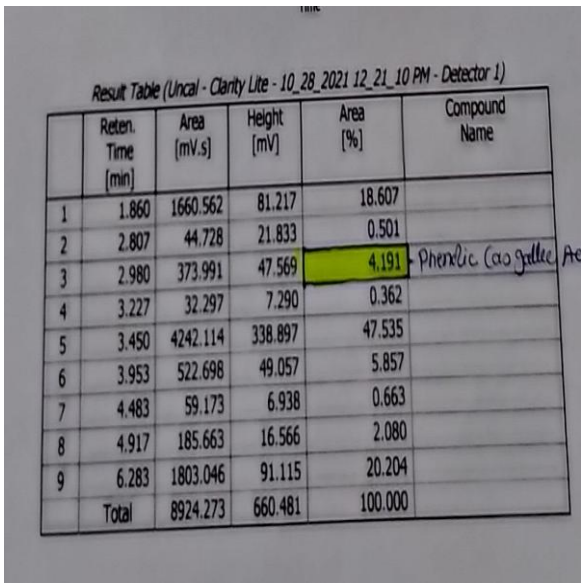




Time

Result Table (Uncal - Clarity Lite - 10_28_2021 11_14_01 AM - Detector 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name
1	2.013	1248.221	72.629	15.142	
2	2.893	29.764	2.592	0.361	
3	3.000	303.639	41.876	3.683	Phenolic Cas gallic A
4	3.190	13.151	0.765	0.160	
5	3.350	25.656	6.688	0.311	
6	3.573	4156.176	291.545	50.417	
7	4.027	6.532	2.257	0.079	
8	4.147	43.833	8.713	0.532	
9	4.253	10.304	0.150	0.125	
10	4.740	31.935	3.355	0.387	
11	5.273	257.684	18.229	3.126	
12	6.870	2116.765	109.752	25.677	
	Total	8243.659	558.552	100.000	



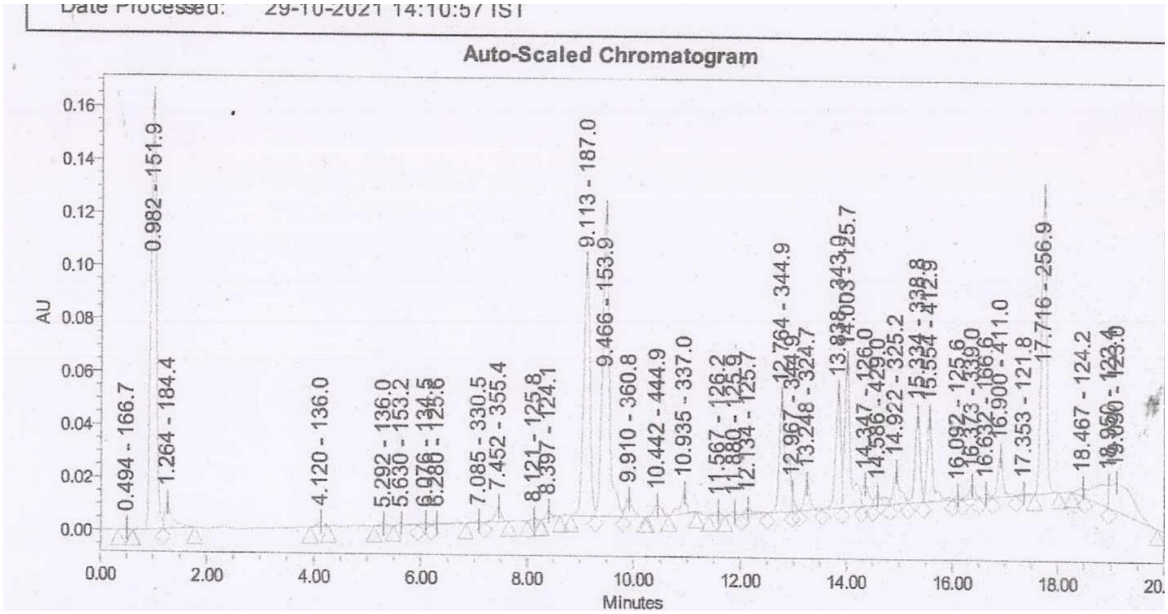
Result Table (Uncal - Clarity Lite - 10_28_2021 12_21_10 PM - Detector 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name
1	1.860	1660.562	81.217	18.607	
2	2.807	44.728	21.833	0.501	
3	2.980	373.991	47.569	4.191	Phenolic Cas gallic Ac
4	3.227	32.297	7.290	0.362	
5	3.450	4242.114	338.897	47.535	
6	3.953	522.698	49.057	5.857	
7	4.483	59.173	6.938	0.663	
8	4.917	185.663	16.566	2.080	
9	6.283	1803.046	91.115	20.204	
	Total	8924.273	660.481	100.000	

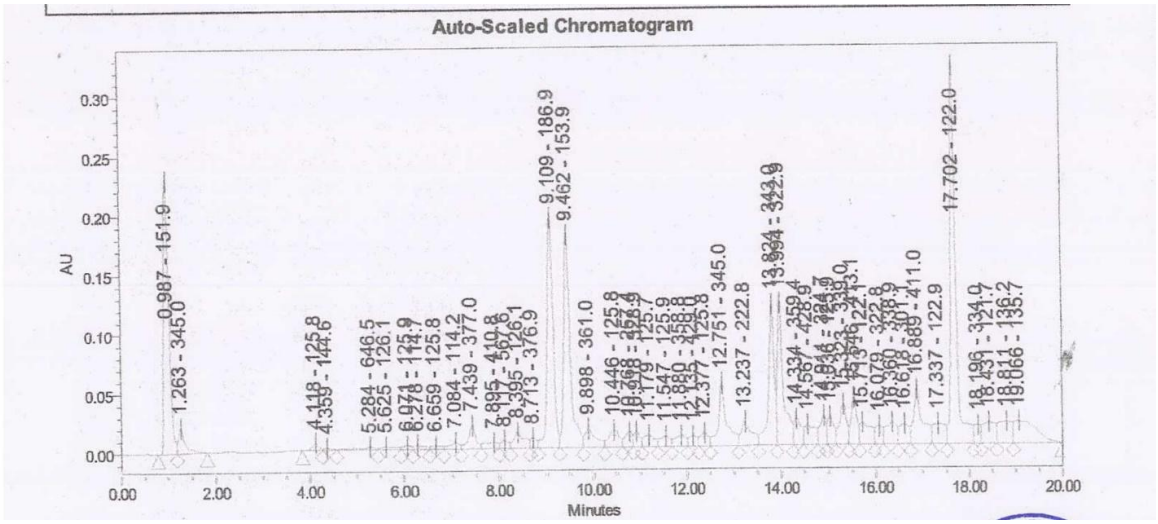
Change sequence
Old Seed TPC(total Phenolic content)
By spectro-photo metric method = 24.31 mg GAE/gm
By HPLC method = 16.69 mg/gm

New Seed TPC(total Phenolic content)
By spectro-photo metric method = 34.23 mg GAE/gm

By HPLC method = 41.191 mg/gm



Fresh Seeds



Stored seeds

IV. Disscusion & Conclusion

The study indicates that the fresh seeds used have more alkaloid and phenolic content than the one store for over a year. Maximum alkaloids and phenols are extracted in methanol. While in other organic solvents, the extraction seems to be very less. The older seeds powder also seems to lose its microbial activity against Bacteria like *E coli* but remains more effective against fungi like *Candida albicans* at same concentration of fresh seeds, Whereas the fresh seed powder was more effective against bacteria than the older seeds powder.

The LCMS graphs also changes in lots of chemical components of the stored and fresh seeds, which can be clearly seen by the changes in retention time of the constituents indicated by the difference in peaks obtained. This study indicates combination of methanol extracts being more effective than water extracts as well as the powder if stored properly retains its antifungal activity, which is also an important property of this plant. This in-vitro study was done for a limited period, so the effect of storage conditions and time period needs to be studied more to find the best way of using the seeds as medicine.

Bibliography

[1]. Samy, R. P., Ignacimuthu, S., & Raja, D. P. (1999). Preliminary screening of ethnomedicinal
[2]. plants from India. *Journal of Ethnopharmacology*, *66*(2), 235-240.
[3]. Abreu, A. C., McBain, A. J., & Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Natural product reports*, *29*(9), 1007-1021.
[4]. Khastgir HN, Duttagupta PC, Sengupta P. Psoralen and Isopsoralen from Psoralea corylifolia. Indian J Appl Chem. 1959;22:82–5.
[5]. Rastogi RP, Mehrotra BN. Vol. 5. Lucknow, CDRI and New Delhi: NISCIR; 1998. Compendium of Indian Medicinal Plants; pp. 703–4.
[6]. Rastogi RP, Mehrotra BN. Vol. 1. Lucknow, CDRI and New Delhi: NISCIR; 2004. Compendium of Indian Medicinal Plants; pp. 332–3.
[7]. Rastogi RP, Mehrotra BN. Vol. 2. Lucknow, CDRI and New Delhi: NISCIR; 1999. Compendium of Indian Medicinal Plants; pp. 567–8
[8]. Rastogi RP, Mehrotra BN. Vol. 3. Lucknow, CDRI and New Delhi: NISCIR; 2001. Compendium of Indian Medicinal Plants; pp. 535–536.
[9]. Qiao CF, Han QB, Mo SF, Song JZ, Xu LJ, Chen SL, et al. Psoralenoside and Isopsoralenoside, two new benzofuran glycosides from Psoralea corylifolia. Chem Pharm Bull. 2006;54:714–6.
[10]. Qiao CF, Han QB, Song JZ, Mo SF, Kong LD, Kung HF, et al. Chemical fingerprint and quantitative analysis of fructus psoraleae by high-performance liquid chromatography. J Sep Sci. 2007;30:813–8.
[11]. Khatune NA, Islam ME, Rahman MA, Baki MA, Sadik G, Haque MA. Pesticidal activity of a novel coumestan derivative isolated from Psoralea corylifolia L. against Tribolium castaneum Herbst. adults and larvae. Pak J Agron. 2002;1:112–5.
[12]. Ruan B, Kong LY, Takaya Y, Niwa M. Studies on chemical constituents of Psoralea corylifolia L. J Asian Nat Prod Res. 2007;9:41–4.
[13]. Bourgaud F, Poutaraud A, Guckert A. Extraction of coumarins from plant material. Phytochem Anal. 2008;5:127–32.
[14]. Matsuda H, Sugimoto S, Morikawa T, Matsuhira K, Mizuguchi E, Nakamura S, et al. Bioactive constituents from Chinese natural medicines. XX. Inhibitors of antigen- induced degranulation in RBL-2H3 cells from the seeds of Psoralea corylifolia. Chem Pharm Bull. 2007;55:106–10.

Sheela Vasu, et. al. "Comparative study of extractive constituents from seeds of Psoralea corylifolia. Linn & its microbial activity." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 15(05), (2022): pp 59-65.