A DISSERTATION ON

"INVESTIGATION OF ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF PSORALEA CORYLIFOLIA SEED EXTRACT AGAINST NON-SMALL CELL LUNG CANCER A549 CELLS".

SUBMITTED TO THE DEPARTMENT OF BIOSCIENCES INTEGRAL UNIVERSITY, LUCKNOW

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FOR THE

DEGREE OF MASTER OF SCIENCE

IN BIOCHEMISTRY

BY,

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M.sc Biochemistry (VI semester)

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UNDER THE SUPERVISION OF

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TO WHOM IT MAY CONCERN

This is to certify that **Miss Alfiya Khan** student of M. Sc. Biochemistry (1V semester), Integral University has completed her four months dissertation work entitled "**Investigation of antioxidant and cytotoxic potential of *Psoralea corylifolia* seed extract against non-small cell lung cancer A549 cells"** successfully. She has completed this work under the guidance of Dr. Irfan Ahmad Ansari. The dissertation was a compulsory part of her M. Sc. degree.

I wish her good luck and bright future.

Dr. Snober S. Mir,

Head

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Alfiya Khan** during the months Jan – May, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by the candidate herself. The thesis entitled is "**Investigation of antioxidant and cytotoxic potential of *Psoralea corylifolia* seed extract against non-small cell lung cancer A549 cells"** is therefore, being forwarded for the acceptance in partial fulfillment of the requirements for the award of the degree of Master of Science in Biochemistry , Department of Biosciences, Integral University, Lucknow, (U.P).

Dr. Irfan Ahmad Ansari

Associate Professor

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Alfiya Khan

CONTENTS:

LIST OF FIGURES AND TABLES:

	cells. The results represented are the mean±SEM of three independent experiments performed in triplicate.	
Figure:10	The images of the untreated and treated lung cancer cell line (A549 cells) showed observable morphological changes under phase contrast microscope.	42

List of Abbreviation:

MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide assay.

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl assay.

FRAP: Ferric Reducing Anti-oxidant Power assay.

DMSO: Dimethyl sulfoxide.

PBS: Phosphate buffered saline.

RPM: Revolutions per Minute

TCA: Trichloroacetic acid

PCD: Programmed Cellular Death

NADPH: Nicotinamide adenine dinucleotide phosphate.

T-BuOOH: Tert-Butyl Peroxybenzoate

CCl4: carbon tetrachloride

WHO: World Health Organization

HPV: Human Papillomavirus

Introduction:

Psoralea *corylifolia* generally known as babchi or buguzhi, is a famous herb, which has been that long used in traditional Ayurvedic and Chinese medicinal drug for its magical significance to cure diverse skin illnesses. Dry fruit of leguminous plant Psoralea *corylifolia* (syn: Cullen corylifolium Linn.) is one of the maximum popular Traditional Chinese Medicine and officially listed in Chinese Pharmacopoeia (Sah, P., *et.al*, 2006). It is an annual herb developing within the bounds of the plains of India (typically happening in tropics and subtropics globally). The plant has biological importance and has been widely exploited glimpses that a long time for its magical impact in opposition to several pores and skin sicknesses, together with psoriasis, leucoderma, and leprosy (Mukherjee, *et.al*, 2002).The name 'Psoralea' is derived from the Greek term 'Psoraleos', which means "affected with itch or with leprosy" (Chopra, *et.al*, 2013).This genus of Psoralea is a major source of various bioactive compounds which belong to the chemical classes of flavonoids, coumarins, furanocoumarins, chalcones, terpenoids, and meroterpenes. Medicinally important compounds procured from Psoralea species are namely - 'psoralen', 'isopsoralen' (Angelicin), 'bakuchiol', 'corylifol', 'psoralidin', 'bavachinin', 'corylifolinin', 'caryophyllene', 'β-farnesene', 'α-pinene', 'camphene' and 'germacrene D' (Bertoli *et.al*, 2004)**.** Some species of Psoralea are poisonous. Species like Psoralea *hypogeae*, Psoralea *esculenta* and Psoralea *macrostachya* of the genus Psoralea have edible starchy roots. There are few species of Psoralea on which pharmacological research reports are available namely *P. bituminosa*, *P. canescens*, *P. corylifolia*, *P. esculenta*, *P. plicata* and *P. glandulosa* .P. *corylifolia* is a medicinal herbs that is commonly known as bavachi, Indian bread root, fountain-bush or scurf pea (Alam *et.al*, 2017, Shilandra *et.al*, 2010, Zhang *et.al*,, 2016).This plant is pharmacologically studied for its chemo protective, antioxidant, antimicrobial, and anti-inflammatory homes. The ethanol extracts of the seeds acts as a medication towards bone fracture, osteomalacia and osteoporosis. The plant is almost entirely used as a tonic or an aphrodisiacal. It belongs to a group of endangered species. It alludes to as "Kushtanashini" meaning Leprosy destroyer (Khushboo, *et.al*, 2010). The call 'Buguzhi' (Fructus psoraleae) certainly comprises of three Chinese words: 'Bu' approach 'to invigorate'; 'Gu' means 'bone' and the third phrase 'Zhi' implies 'fats'. The Chinese name of the herb shows the justification of the herb to provide fat for the revitalizing bones. The plant have blood purifying properties and therefore used to deal with boils, itching eruptions or purple papules, ringworm-contamination, substantial eczema, tough and discolored dermatosis

with fissures and scabies (Khare, *et.al*, , 2004). The essential oil from plant allegedly has a strong impact on Streptococcal contamination of the pores and skin (Khushboo *et.al*, 2010).The plant has its application in each method: - inside and out. The seed oil can be implemented externally towards many pores and skin issues. It is used in disease characterized by using hypo pigmented lesions which include leucoderma and psoriasis both through nearby software and oral therapy. Psoralea *corylifolia* seed extracts depicts the characteristics such as anti-hyperglycemic, anti-depressant, anti-tumor, anti-bacterial and anti-oxidant property (Steven, *et.al*,1993)**.**The oil obtained from the plant is used in opposition to the pores and skin Streptococci. It is used in opposition to the ailment vitiligo that's characterized with the aid of the advent of patches at the skin due to much less pigmentation. The end result is sour in flavor, can prevent vomiting, treatment difficulty in micturition, remedy piles, bronchitis and anaemia and improve complexion (Joshi, 2000)**.** It is beneficial in opposition to dermatitis, inflammatory disorders, muco-membranous issues and edematous troubles of the pores and skin. It enables in relieving boils and eruptions of the pores and skin. The plant performs a critical part to treat itching, eruptions, ringworm, full-size eczema with thickened dermis, itching purple papules, dermatosis with fissures and scabies, hard and discolored dermatosis. Seeds are beneficial towards nauseous problems and are also given in scorpion-sting and snake bite. The powder of seeds is used as an antihelminthic, laxative, diuretic, stomachic, stimulant, aphrodisiac, diaphoretic and for recovery wounds (Khushboo, *et.al*, 2010; Khare, *et.al*, 2004; Steven, *et.al*, 1993; Joshi, 2000; Sharma, *et.al*, 2000)**.**The important components psoralen and isopsoralen are identified to possess anti-bacterial, anti-viral and anti-tumor properties(Liu *et.al*,, 2004)**.** Psoralen and isopsoralen compounds acquired from Psoralea species are also below trials towards syndromes like AIDS (Anis *et.al*, 2005; Bhattacharjee, 1998; Duke, 2009)**.** The seeds are used for curing diverse problems together with cough, asthma, nephritis, alopecia areata, menstruation, uterine disorders and haemorrhages (Qiao, *et.al*, 2007**)**. Psoralea species are tough to propagate because of terrible seed-germination and excessive seedling-mortality (Mitter *et.al*, 1993)**.**The crude extracts of seeds are used inside the treatment of febrile sicknesses, impotence, spermatorrhea, premature ejaculation, decrease lower back pains, incontinence, enuresis, pollakiuria, and bloodless symptoms in the waist and knees (Chopra *et.al*, 1956; Lin *et.al*, 2007, Zhao *et.al*, 2005a, Zhao *et.al*, 2005b)**.**

Cancer is an age-based disease (De Pinho, *et.al*, 2000) sustained by using five pointers in cell physiology that propel the progressive change of standard differentiated cells into

diverse malignancy states (Demetrius, *et.al*, 2010) autonomous extension replication in the absence of advancement signals, insensitivity to anti-development alerts, apoptosis illusion of programmed cell death, angiogenesis-the initiation of the increment of new blood vessels; descent and metastasis. Cancer cells may be taken into consideration as self sustaining units which have an impaired ability to keep the metabolic stability of the organism wherein they live. Genetic injury that initiates in a regular discrete cell drives toward into an unusual cell so that it will originate a carcinogenic manner, accompanied via the boom of a rigid tumor mass. Different forms of rigid tumors are described as sarcomas, carcinomas and lymphomas (Machado, *et.al*, 2011). Carcinogenic technique is characterized through the deregulation of regular cell metabolic pathways disrupting particularly each cellular cycle arrest and programmed cellular death (PCD) including apoptosis, autophagy, and necroptosis (Chow, *et.al*, 2010; Christofferson, *et.al*, 2010; Malumbres, *et.al*, 2003) including a cell reprogramming. Under ordinary conditions, the stability among cell proliferation and cell loss of life stays tightly regulated to make sure tissue homeostasis (Gérard, *et.al*, 2014; Ortiz, *et.al*, 2012). Tumors do not possess a risk to life until they compress vital structures or have a few physiological interest but there is proof that a benign tumor can turn out to be in a premalignant tumor and finally becoming malignant (Shlush, *et.al*,. 2015 ; Panwar, *et.al*, 2014). All of the cells of the complete body are continuously uncovered to exceptional endogenous and exogenous retailers that induce somatic mutations in a without delay or incidental way (Petljak, *et.al*, 2016; Terabayashi, *et.al*, 2018). Cancer turn of events takes three specific phases: initiation, promotion and progression (Gordon, *et.al*, 2018). Tumor initiation is idea to be the end result of a genetic alteration mainly to anomalous proliferation of a single cellular. Cell proliferation then results in the outgrowth of a populace of clonally derived tumor cells. Tumor progression keeps as extra mutations arise inside cells of the tumor populace. Within those levels is viable to explain the characteristics received by most cancers cells from the induction of mutations (mutagenesis) to unfold the cancer cells (metastasis).

Metastasis is an convoluted process consisting of multiple steps which embark on with detachment, accumulating, and motion of cancerous cells culminating in attachment to endothelial cells perceived with increase of most cancers cells at unique sites (Martin, *et.al*, 2013) ; Adnan, *et.al*, 2017). Metastasis is the direct cause for cancer related death due to objection to diverse cytotoxic agents including apoptosis. The reason of death and anguish in patients with metastatic cancer where chemotherapy drugs are doomed in

efficaciously exterminate the cancer cells without damaging healthy cells (Redig, *et.al*, 2013). Metastasis is activated via angiogenesis, the proliferation of a complex system of blood vessels that insinuate oneself into cancerous tissue, delivering nutrients and oxygen (Zetter, *et.al*, 1998).

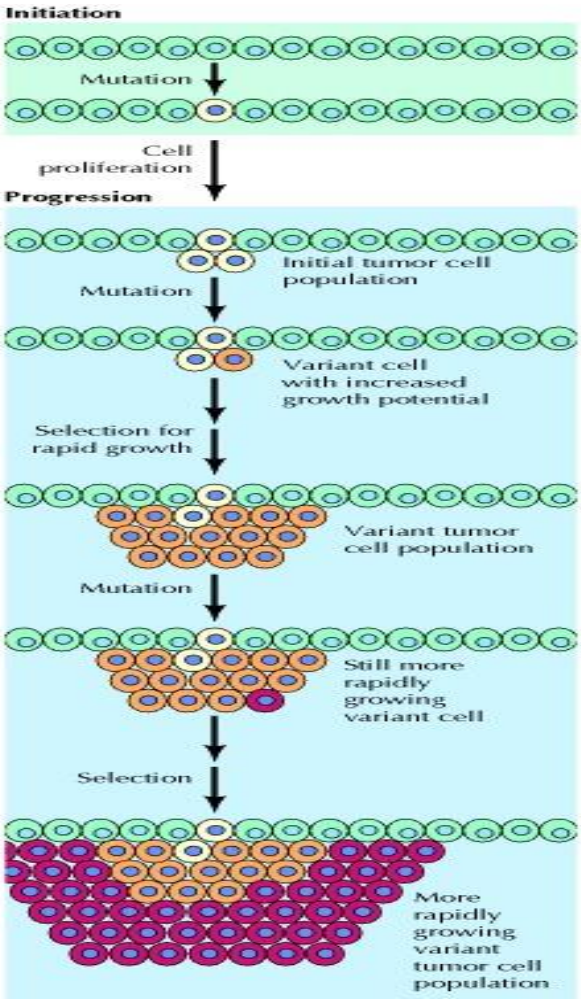


Figure 1: Cancer progression steps: Initiation, Promotion and Progression.

DNA damaging of a normal cell (initiation) results into uncontrolled growth and proliferation of the inaugural cell (promotion) with ongoing growth and intrusion of tissue of origin of the cancer progression steps.

Review of literature:

Psoralea corylifolia is a well-known traditional medicinal plant utilized from ancient era for treatment of various ailments. It is widely distributed and an important part of therapeutics in Ayurveda and in Chinese medicines. The plants of Leguminosae are widely distributed, and in terms of number of specie, it is one of the largest terrestrial families of plants after Orchidaceae and Asteraceae (Stevens, 2015).

Description of the plant:

It is a small, erect, annual herb developing up to 60–120 cm in top at some stage in sandy, loamy plains of Central and East India (Joshi, *et.al*, 2000 ;Pole, *et.al*, 2006)**.**Seeds are brownish black in coloration, oblong, and flattened. Pods are small, oval, mucronate, oblong, fairly compressed and chocolate to black in color. Das defined the seeds as kidney appearance, 2–4 mm long, 2–3 mm extensive, and 1–1.5 mm thick, hard, clean, ex-albuminous with straw-colored testa, with an agreeable fragrant odor and a smelly-sour flavor (Chopra, *et.al*, 1958)**.**They have grooved and gland-dotted stems (Khushboo, *et.al*, 2010)**.** Leaves are easy, widely elliptic, rounded, and mucronate at apex, clothed with white hairs on each surfaces, blanketed with numerous black dots, 5 primary nerves springing from the base (Khushboo, *et.al*, 2010). Flowers are dense, corolla is yellow or bluish crimson (Khushboo, *et.al*, 2010) and axillary in aestivation and racemes are 10–30 flowered (Khushboo, *et.al*, 2010)**.** Flowering time starts from August and ends in December. Fruit is smaller in size, 5 mm in length, sub globular in shape, barely compressed, black pitted, beaked without hairs, indehiscent and one-seeded pod adhering to the pericarp. The roots of P. *corylifolia* have been investigated for bioactive compounds. It turned into the discovery that furanocoumarins psoralen and isopsoralen isolated from a petrol ether extract have been chargeable for the anti-feedant pastime towards instar Spodoptera litura larvae (Sah *et.al*, 2006).

Classification of Psoralea plant:-

The classification of plant details are (Sah, *et.al*, 2006)**;**

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Rosales

Family: Leguminosae

Subfamily: Papilionaceae

Genus: *Psoralea*

Species: *corylifolia* Linn.

Whole plant: The extraction protocol of the whole plant of *P. corylifolia* was extracted with organic solvents namely ethanol. The isolation measures lead the way to the purification of bioactive compounds such as psoralen, isopsoralen, corylifolin, corylin, and psoralidin (Gupta, *et.al*, 2013). Peng and his colleague acquired a new compound spotted out as Neo-psoralen from the whole plant of *P. corylifolia* in the year 1996 and enlightened its structure according to its chemical indications and spectroscopic analysis (Chaudhuri, 2015; Gupta *et.al*, 2013).

Seeds: Sen and his colleague extracted the seed oil from the seeds of *P. Corylifolia* and determined that the oil is unsaponifiable with boiling factors 180–190 °C. Chopra and Chaterjee in the year 1927 identified an essential oil, a fixed oil and resins of dark brownish color with some relics of alkaloids in *P. corylifolia* (Chopra *et al.,* 2013). A very crucial pharmacological compound designated as bakuchiol has also been biosynthesized in 1983, and it came to the conclusion that it is a derived product of phenyl propane pathway (Banerji *et.al*, 1983). Bisbakuchiols A and B structures were estimated and it was observed that dimeric monoterpenoid framework comprises two monoterpenes, which are linked with the help of a dioxane bridge (Wu *et al.,* 2007).

Fruits: The sticky and oily pericarp makes the fruit of *P. corylifolia* and chemical research found out a few comparable compounds as segregated from seeds. Six different compounds were isolated from the fruit of *P. corylifolia* are isopsoralen, psoralen, sophoracoumestan A, neobavaisoflavone, daidzein, and uracil, have been stated from the dehydrated fruits of *P. corylifolia* (Ruan, *et.al*, 2007). Raun and his co-workers have secluded seven compounds from *P. corylifolia* fruit and organised the shape of compounds after spectroscopic analysis. The compounds obtained were corylinin, psoralen, neobavaisoflavone, sophoracoumestan A, uracil, and daidzin (Ruan *et al.,* 2007).

Distribution/ Habitat: It grows at some point of the plains of India, especially in the semi-arid regions of Rajasthan and Eastern districts of Punjab, adjacent Uttar Pradesh. It is

also discovered at some stage in India in Himalayas, Dehra Dun, Oudh, Bundelkhand, Bengal, Bombay, some valley in Bihar, Deccan and Karnataka(Sah, Agarwal, & Garg, 2006; Sharma, Yelne, Dennis, Joshi, & Billore, 2000)**.** This plant is likewise broadly dispensed inside the tropical and subtropical regions of the world, especially China and Southern Africa (Qiao, *et.al*, 2006)**.**

Propagation and Cultivation: -The plant flourishes well in areas with low to medium rainfall during the summer season and on a variety of soils ranging from sandy, medium loam to black cotton in dry tropical regions of India. The germination percentage can be significantly elevated by sowing the seeds in the course of summer time (March–April) and leaving them inside the heat of the soil. Mechanical puncturing of the seed coverings or presowing remedy with concentrated sulfuric acid for 60 min has additionally been found powerful in breaking the dormancy of the seeds and growing the germination percent drastically. The crop takes 7–8 months to attain maturity. As seeds continue to mature constantly, 4–5 pickings are generally taken between December and March. Clonal propagation of P. *corylifolia* via shoot tip and axillary bud tradition is accomplished. Survival price on swap over area turned into 95% (Khare, *et.al*, 2004)**.** Unfavorable factors for the cultivation of the plants are: Lengthy gestation period, low germination percent and viability of the seeds and delicate field management.

Parts Used: Leaves, seeds, seed oil and roots were used (Sharma, *et.al*, 2000). Roots are utilized in dental aids. Leaves are used towards diarrhea. Fruits are used as aphrodisiacal, laxative and are used in opposition to psoriasis, leucoderma, leprosy and inflammatory problems of the pores and skin. *Psoralea* is a single seed plant. The seeds neither have starch nor any endosperm but have oily texture, (Krishnamurthy, 1969). The seeds mature slowly, and hence their collection can be accomplished 4–5 times from December to March (Sharma *et al.,* 2000).

Active Compounds:-

The plant extracts were suggested to own antibacterial, antitumor, antioxidant, anti inflammatory, antifungal and immunomodulatory interest. A wide range of chemicals inclusive of psoralen, isopsoralen, bakuchiol, psoralidin, bakuchalcone, bavachinin, flavones, risky oils, lipids and so on are located in one of a kind parts of the plant. The powder and extracts of *Psoralea corylifolia* L. had been examined in lard at 100 °C by using the oxidative stability device (OSI) and were found to have sturdy antioxidant consequences. Six compounds: bakuchiol, psoralen, isopsoralen, corylifolin, corylin and

psoralidin have been remoted from the herb and identified via UV, IR and Mass, 1H and 13C NMR spectra and melting factor. Their antioxidant activities have been investigated personally and compared with butylated hydroxytoluene (BHT) and α-tocopherol via the OSI at 100 °C. The outcomes showed that bakuchiol, corylifolin, corylin and psoralidin had robust antioxidant sports and particularly psoralidin (stronger antioxidant property than BHT), however psoralen and isopsoralen had no antioxidant sports at 0.02% and 0.04% tiers. The antioxidant sports of the compounds lower within the following order: Psoralidin> BHT > α-tocopherol > bakuchiol >corylifolin>corylin>isopsoralen > psoralen (Jiangning *et al.,* 2005).

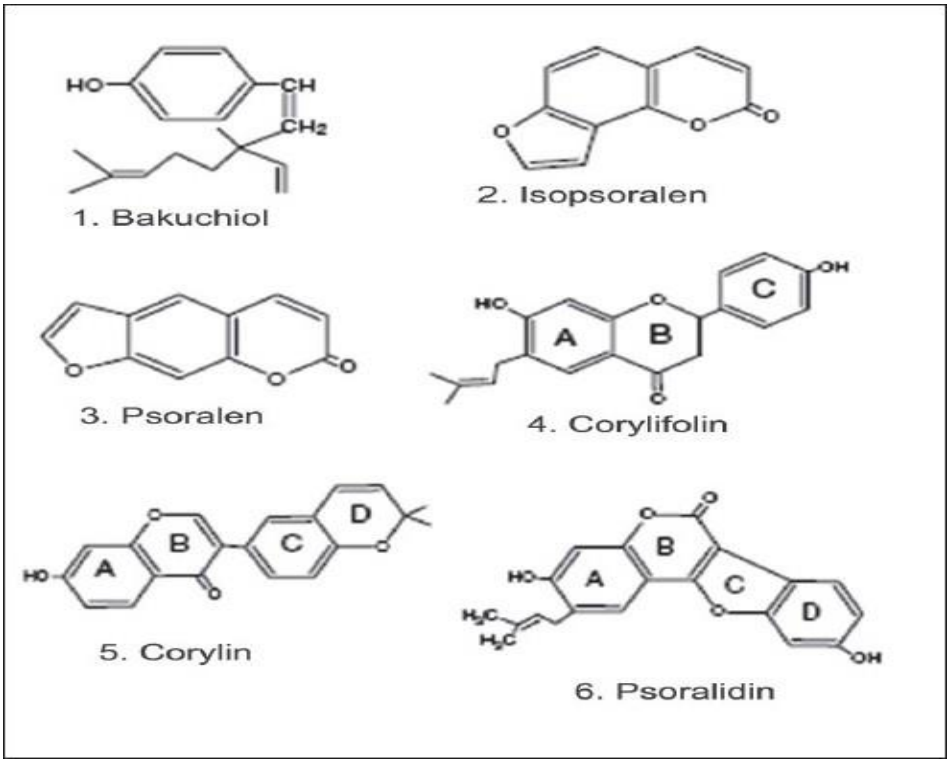


Figure 2: Structure of major constituents of Psoralea *corylifolia* (Sharma, *et.al*, 2000).

Anti-oxidant activity of Psoralea: A meroterpene and 4 flavonoids had been isolated from the seeds of *Psoralea corylifolia* as antioxidative components. Their systems have been elucidated by way of spectral data and identified as;

- Bakuchiol
- Bavachinin
- Bavachin
- Isobavachin
- isobavachalcone

They inhibited NADPH, ascorbate, t-BuOOH- and CCl4-brought on lipid per oxidation in microsomes. They additionally averted NADH-dependent and ascorbate-brought on mitochondrial lipid per oxidation. Bakuchiol became the maximum effective antioxidant in microsomes and the inhibition of oxygen consumption brought about by means of lipid per oxidation became time-established. It protected human red blood cells in opposition to oxidative haemolysis. These phenolic compounds in P. *corylifolia* had been shown to be powerful in shielding organic membranes towards diverse oxidative stresses (Shaikh, *et.al*, 2021).

Phytochemistry:-

Phytochemical analysis of *Psoralea corylifolia* showed the presence of β-sitosterol, terpenoids, phenolic compounds, saponins, glycosides, tannins, chalcones derivatives, coumestans, coumarins, monoterpenes and benzofuran glycosides.Chopra et al located that the seeds include a vital oil (0.05%), a nonvolatile terpenoid oil, a dark brown resin (8.6%), and lines of alkaloidal substance**.** Dymock stated that the seeds incorporate 13.2% of extractive matter, albumin, sugar, ash 7.4%, and lines of manganese**.** Sen. et al determined that the seeds contained an unsaponifiable oil having the system C17H24O, boiling among 180 and 190°C, a yellow acid substance C40H45O10 from the alcoholic extract and a o-methyl glycoside having a Melting Point of about 105–107°C, containing 4 (OH) groups. The end result of P. *corylifolia* consists of a sticky oily pericarp (12% of the seed), a tough seed coat and kernel. A pigment (probably a hydroxy flavone), a monoterpenoid phenol named bakuchiol (C18H24O, B.P. 145–147°C), brown constant oil (10%) and raffinose and coumarin compounds were also determined inside the seeds (Chopra, *et.al*, 1958)**.** The critical oil consists of limonene, α-elemene, γ-elemene, β-caryophylenoxide, four-terpineol, linalool, geranylacetate (Khushboo, P. S., Jadhav, et.Al, 2010)**.** The essential oil consists of linalool, α-elemene, 4-terpineol, γ-elemene, limonene, β-caryophylenoxide, geranylacetate, active component psoralen (identical with ficusin; C11 H6 O3, m.p. 161-162°C). Petroleum ether extract of the seeds are known to own resin acids (21.5%) and glycerides of linoleic acid, myristolic acid, oleic acid, stearic acid, myristic acid, linolenic acid and palmitic acid. Phytochemical reported are : psoralen, psoralidin, corylin, bakuchiol, isopsoralen and corylifolin61, 4-methoxy flavone, Monoterpenoid- bakuchiol A and B and (S)-Bakuchiol, Bavachin, bavachinin, bavachalcone, corylifol A, B and C, neobavaisochalcone, isoneobavachalcone, isobavachalcone, 8-prenyl Diadzein, brosimacutin, bakuchalcone and erythrinin. The various phytochemical isolated are 12, 13-dihydro-12,13-epoxy bakuchiol, psoracorylifol

A-E. Angelicin and cyclobakuchiols A and B have been reported from dichloromethane extracts of Psoralea *corylifolia.* Bavachin, bavachinin, 6-prenyl naringenin, 3-hydroxy bakuchiol, γ-cadinene, diadzein, genistein, psoralester, psorachromene, 7-methoxybavachin, chromenoflavone, 4-hydroxylonchocarpin, bavachalcone, Bavachin, corylifolinin, bavachinin are some other phytoconstituents documented from *Psoralea corylifolia*. The first purest compound obtained from *Psoralea corylifolia* is psoralen by Jois and his coworkers in the year 1933**.** The secondary metabolites obtained from the genus *Psoralea* include flavonoids, coumarins, phenols, benzofuran, benzopyrans, quinine, sesquiterpenoids, triterpenoids, steroids, etc.

Bioactive Compounds:-The bioactive compounds isolated from *P. corylifolia* with reported activities [Table 1]:

Number	Compound	Chemical nature	Part of the plant	Activity&References
1.	Angelicin	Furanocoumarins	Seeds	Antibacterial (Khatune et al., 2004)
2.	Aryl coumarin	Coumarin	Seeds	Anticancer (Limper et al., 2013)
3.	Astragalin	Flavonoids	Seeds	Antioxidant (Zhang et al., 2016)
4.	Bakuchiol	Meroterpene	Seeds/Fruit	Anti-acne (Iwamura et al., 1989) Antibacterial (Katsura et al., 2001; Newton et al., 2002) Antifungal (Newton et al., 2002), (Hosamani et al., 2012; Lau et al., 2010 Lau et al., 2014; Prasad et al., 2004; Savoia, 2012

				Srinivasan & Sarada, 2012; Yang et al., 2006) Retinal regeneration (Sec et al., 2013) Anti-aging (Seo et al., 2013) Estrogen receptor agonist, Postmenopausal symptoms (Lim et al., 2011) Anti-diabetic (Behloul& Wu, 2013) Lymph angiogenesis inhibition (Jeong et al., 2013) Anticancer (Chen et al., 2010; Li et al., 2016)
5.	Bavachinin	Flavone	Seeds	Antibacterial (Khatune et al., 2004) Estrogen receptor agonis

				(Lim et al., 2011) Lymphangiogenesis inhibition (Jeong et al., 2013) osteoporosis (Liu et al., 2014) Anti-Alzheimer (Chen et al., 2013) Carboxyl esterase inhibitors (Li et al., 2015)
6.	Bakuisoflavone	Flavone	Fruit	Antibacterial (Siva et al., 2015)
7.	Bakuflavanone	Flavone	Fruit	Antibacterial (Siva et al., 2015)
8.	Bavachin	Flavonoid	Seeds/fruit	Osteoblast (Miura & Nishida, 1996)
9.	Bakuchicin	Coumarin	Seeds	Topoisomerase inhibitor (Sun et al., 2003)
10.	Bavachalcone	Chalcone	Seeds	Anticancer (Shan et al., 2014) CVS protective effect (Dang et al., 2015)
11.	Bavachinone A	Flavonoid	Fruit	Antibacterial (Won et al., 2015)
12.	Bavachinone B	Flavonoid	Fruit	Antibacterial (Won et al., 2015)
13.	Bavacoumestan C	Flavonoid	Fruit	Antibacterial (Won et al., 2015)

14.	Corylifolinin	Chalcone	Seeds	Antibacterial (Khatune et al., 2004) Carboxylesterase inhibitors (Sun et al., 2016)
15.	Corylifols	Prenyl Flavonoid	Seeds	Antibacterial (Yin et al., 2004)
16.	Corylifol A	Flavonoid	Seeds/fruit	Carboxylesterase inhibitors (Li et al., 2015)
17.	Corylifol B	Flavonoid	Seeds	Carboxylesterase inhibitors (Li et al., 2015)
18.	Corylifol C	Flavonoid	Seeds	Protein kinase inhibition (Limper et al., 2013) Anticancer (Limper et al., 2013)
19.	Corylifol D	Flavonoid	Seeds	Anticancer (stomach; Yang et al., 1996; Teschke et al., 2014)
20.	Corylifol E	Flavonoid	Seeds	Anticancer (stomach; Yang et al., 1996; Teschke et al., 2014)
21.	Coryfolin	Flavonoid	Whole plant	Antioxidant, anti-diabetic (Behloul& Wu, 2013)
22.	Corylin	Flavonoid	Whole plant	Osteoblast (Miura & Nishida, 1996; Wang et al., 2001) Anticancer (Shan et al., 2014)

				Carboxylesterase inhibitors (Sun et al., 2016)
23.	Coryaurone A	Flavonoid	Fruit	Antibacterial (Won et al., 2015)
24.	Dadzin	Isoflavonoid	Fruit	Antioxidant (Shinde et al., 2010)
25.	Daidzein	Isoflavonoid	Fruit	Antioxidant (Shinde et al., 2010) Antidiabetic (Behloul& Wu, 2013) Topoisomerase inhibitor (Sun et al., 2003)
26.	Dihydroxy coumestans	Essential oil component	Seeds	Insecticidal, genotoxic (Khatune et al., 2002; Dua et al., 2013)
27.	Genistein	Isoflavone	Fruit	Anti-diabetic, anti-obesity (Behloul& Wu, 2013), antioxidant (Shinde et al., 2010)
28.	Hydroxy bakuchiol	Meroterpene	Seeds	Lymph angiogenesis inhibition (Jeong et al., 2013)
29.	Hydroxypsoralen ol A	Flavonoid	Fruit	Antibacterial (Won et al., 2015)
30.	Hydroxypsoralen ol B	Flavonoid	Fruit	Antibacterial (Won et al., 2015)
31.	Isobavachalcone	Chalcone	Seeds	Estrogen receptor agonis

				(osteoporosis; Lim et al., 2011) Neuroprotective (Lee et al., 2015) Lymphangiogenesis inhibition (Jeong et al., 2013) Anti-Alzheimer (Chen et al., 2013) Carboxylesterase inhibitors (Li et al., 2015)
32.	Isobavachin	Flavonoid	Seed/fruit	Osteoblast (Li et al., 2014)
33.	Isopsoralen	Furanocoumarins	Whole plant	Antiprotozoal (Song et al., 2015)
34.	Neobavaisoflavone		Seeds	Antibacterial(Khatune et al., 2004)
35.	Psoralen	Furanocoumarins	Whole plant/root	Leucoderma, psoriasis (Kim et al., 2013) Anticancer (Hao et al., 2014), antioxidant (Chen et al., 2011), anti-Alzheimer (Somani et al., 2015), Collagengenesis (Xu et al., 2015)

36.	Psoralidin	Coumarin	Whole plant/seed	Estrogen receptor modulator (Liu et al., 2014; Lim et al., 2011 Antioxidant (Wang, Yin, Zhang, Peng, & Kang, 2013), antibacteria (Khatune et al., 2004) Anti-diabetic (Behloul& Wu, 2013), Antiprotozoal (Song et al., 2015) Anticancer (Hao et al., 2014; Limper et al., 2013; Yang et al., 1996), anti-depressan (Farahani et al., 2015)
37.	Psoracorylifol D	Flavonoid	Seed	Lymphangiogenesis inhibition (Jeong et al., 2013)
38.	Psoracoumestar	Coumestans	Seeds essential oi	Anti- cancer (Limper et al., 2013)

Cancer: Cancer can begin almost anywhere in the human body, which is made from trillions of cells. Normally, human cells develop and multiply (through a technique referred to as cell department) to form new cells because the body needs them. When cells develop vintage or come to be broken, they die, and new cells take their location. Sometimes this orderly manner breaks down, and bizarre or broken cells grow and multiply once they shouldn't. These cells may additionally shape tumors that are lumps of tissue. Tumors may be cancerous or not cancerous (benign). Cancerous tumors spread into, or invade, nearby tissues and may tour to distant places within the frame to shape new tumors (a process known as metastasis). Cancerous tumors can also be known as malignant tumors. Many cancers shape solid tumors, but cancers of the blood, such as leukemia's, generally do not. Benign tumors do now not unfold into, or invade, close by tissues. When eliminated, benign tumors generally don't develop lower back, whereas cancerous tumors sometimes do. Benign tumors can on occasion be quite huge, but.

Some can purpose critical symptoms or be life threatening, which includes benign tumors inside the mind **(**Alba-Ruiz, *et.al*, 2013)**.**

How does cancer develop:-Cancer is a genetic sickness that is caused by adjustments to genes that manage the way our cells function, in particular how they develop and divide? Genetic adjustments that purpose cancer can show up due to the fact **(**Alba-Ruiz, *et.al*, 2013)**.**

- Chemicals in tobacco smoke and ultraviolet rays from the sun.
- They had been inherited from our mother and father.
- Of damage to DNA because of harmful materials inside the environment, inclusive of the errors that arise as cells divide.

Each man or woman's most cancers has a unique combination of genetic changes. As the most cancers keeps growing, extra modifications will arise. Even inside the equal tumor, specific cells may additionally have one of kind genetic changes.

Genes causing cancer: The genetic changes that make a contribution to cancer generally tend to affect three important styles of genes—proto-oncogenes, tumor suppressor genes and DNA repair genes. These modifications are on occasion called "drivers" of most cancers **(**Alba-Ruiz, *et.al*, 2013)**.**

1. **Proto-oncogenes** are involved in regular cell boom and division. However, when those genes are altered insure methods or are extra energetic than regular, they may grow to be cancer-inflicting genes (or oncogenes), permitting cells to grow and survive when they must not.
2. **Tumor suppressor** genes are also concerned in controlling cell boom and department. Cells with assured changes in tumor suppressor genes may additionally divide in and out of control way.
3. **DNA repair genes** are apprehensive in solving broken DNA. Cells with mutations in those genes tend to develop additional mutations in other genes and changes of their chromosomes, along with duplications and deletions of chromosome components. Together, those mutations may additionally bring about the cells to emerge as cancerous.

Cancer spreading: A cancer that has unfolded from the region where it first formed to every other area within the frame is known as metastatic most cancers. The method through which most cancers cells unfold to other parts of the body is referred to as

metastasis. Metastatic cancers have the identical name and the equal form of cancer cells as unique or primary. For example, breast cancer that bureaucracy a metastatic tumor within the lung is metastatic breast cancers and no longer lung cancer **(**Alba-Ruiz, *et.al*, 2013)**.**

Types of cancer: Types of cancer are typically named for the organs or tissues in which the cancers form. For example, lung cancer starts within the lung, and brain cancer starts inside the brain. Cancers additionally can be described with the aid of the kind of cells that fashioned them, which include an epithelial cell or a squamous cell. Here are a few categories of cancers that start in particular varieties of cells (Dubchak, *et.al*, 2010)**:**

- **Carcinoma: -**Cancer that starts inside the skin or in tissues that line or cowl internal organs “pores and skin, lung, colon, pancreatic, ovarian cancers," epithelial, squamous and basal cellular carcinomas, melanomas, papillomas, and adenomas.
- **Sarcoma:** Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or different connective or supportive tissue -- "bone, soft tissue cancers," osteosarcoma, synovial sarcoma, liposarcoma, angiosarcoma, rhabdosarcoma, and fibrosarcoma.
- **Leukemia :** Cancer that starts off evolved in blood-forming tissue along with the bone marrow and reasons massive numbers of extraordinary blood cells to be produced and input the blood -- "leukemia," lymphoblastic leukemia’s , myelogenous leukemia’s , T-cell leukemia, and bushy-mobile leukemia.
- **Lymphoma and myeloma:** Cancers that start within the cells of the immune gadget -- "lymphoma," T-cellular lymphomas, B-mobile lymphomas, Hodgkin lymphomas, non-Hodgkin lymphoma, and lymphoproliferative lymphomas.
- **Central Nervous System Cancer:** Cancers that start in the tissues of the brain and spinal twine - "brain and spinal cord tumors," gliomas, meningiomas, pituitary adenomas, vestibular schwannomas, number one CNS lymphomas, and primitive neuroectodermal tumors.
- **Melanoma: -**Melanoma is a type of cancer that begins in cells that end up melanocytes, which are specialized cells that make melanin (the pigment that offers skin its coloration). Most melanomas form at the pores and skin, but melanomas can also shape in other pigmented tissues, including the attention.

Death rates: Age-adjusted demise costs (deaths in keeping with 100,000 populations) for particular varieties of tumors have changed extensively over the years. In 1996, for the primary time on account that information started out being compiled, most cancers

deaths inside the United States decreased (nearly 3 percent), and the declines continued through the primary decade of the twenty first century. Worldwide, however, dying quotes from cancer were on the upward thrust. The World Health Organization (WHO) projected that 13.1 million people globally could die from most cancers in 2030.In the United States and sure different evolved nations, decreases in death charges from most cancers may be attributed to successes of therapy or prevention. For example, a discount in the wide variety of deaths due to lung cancers has been attributed to warnings that have altered cigarette-smoking habits. Therapy has greatly lessened mortality from Hodgkin ailment and testicular most cancers, and it also has improved the chances of surviving breast cancers. Preventive measures have played a primary position within the decrease of cancer mortality as properly. For instance, colonoscopy, which is used to discover early asymptomatic cancers or premalignant growths (polyps) in the colon, has contributed to declines in death fees from colon cancer. Routine Pap smear, an exam used to display screen for carcinoma of the uterine cervix, has led to a downward trend in mortality determined for that sickness. The identification of certain varieties of HPV as the causal agents of cervical most cancers has progressed cervical cancer screening programs with the aid of permitting samples obtained from asymptomatic girls to be tested for the presence of dangerous viral kinds that would later provide upward thrust to most cancers. The effectiveness of preventative measures for cervical most cancers is notion to be greatly elevated by the supply of HPV vaccines together with Gardasil, which turned into accredited for the immunization of young ladies and boys prior to their becoming sexually energetic**.**

Cancer is one of the most deadly diseases in the global. Cancer specially takes place due to gene mutations, and the situation is worsened via different carcinogenic dealers. Both gene mutations and carcinogenic marketers then affect and change the cellular capabilities and metabolism, and thereby, the replication and spread of cancer cells are out of control. The cancer cells grow and multiplicate rapidly, as a consequence crowding the other regular cells (Hassanpour, *et.al*, 2017)**.** The most cancers cells also attack and consume the normal cells, on account of that cancer cells require big amounts of biomaterials for cell division. For this reason drastic weight loss with lumps at the most cancers cells location is usually determined in most cancers sufferers (Kotecha, *et.al*, 2016)**.** It was concluded that the quantity of fellow sufferers (51%) is barely higher than the wide variety of women patients (49%). Lung, prostate, and colorectal cancers make a contribution to 46% of most cancers instances in guys, at the same time as lung, breast,

and colorectal cancers make a contribution to 50% of most cancers instances in girls (Siegel, *et.al*, 2020)**.** The number of most cancers and mortality cases has been kept increasing, and it's far expected that world cancer cases will increase to 23.6 million in near future (Siegel, *et.al*, 2021)**.** The studies on anticancer drug layout and development is an pressing need, because humans are suffering and the wide variety of dying instances is unstoppable until active, selective, and green anticancer tablets are found (Blackadar, *et.al*, 2016; Carugo, *et.al*, 2019; Olgen, *et.al*, 2018) . P53 is a major responsive tumor suppressor gene that features to govern cellular cycle arrest and induces apoptosis (Roos, *et.al*, 2013)**.** P. Corylifolia leaves comprise remarkably high concentrations (greater than 2 g according to Kg dry weight) of genistein (an anti-cancer metabolite) (Siva, *et.al*, 2015). The compounds psoralen (Kamboj, *et.al*, 2011) and isopsoralen (Agarwal, *et.al*, 2006) induced apoptosis in carcinoma lines KB, KBv200 (vincristine resistance subline of KB) and human erythroleukemia cell K562 and K562/ADM (doxorubicin resistance subline of K562). Both the compounds caused apoptosis in these cells for this reason, confirming their anti-cancer ability. Psoralen whilst subjected to human hepatocarcinoma cells, showed its inhibitory pastime by way of inducing the mechanism of apoptosis. *Psoralen* became able to inhibit the increase of SMMC-7721 cells in a dose- and time-based manner and had a robust proapoptotic impact on these cells (Jiang, *et.al*, 2014; Khan, *et.al*, 2015). The bioactive compounds 7, 2', 4'-trihydroxy-3-arylcoumarin (Khan, S., Iqbal, et.Al, 2015), Psoracoumestan (Wang, *et.al*, 2012) and corylifol C (Pole, S., 2006) from P. *corylifolia* confirmed robust anti-cancer potential by inhibiting the enzyme MAPK/ERK kinase phosphorylation and inducing apoptotic cell death (Limper, *et.al*, 2013).

A plant extract is a vital substance with perfect properties removed from the tissues of a plant, frequently through treating it with a solvent, to be used for a selected reason. The term "bioactive compounds" is generally referred to as biologically large chemical compounds however no longer set up as important vitamins (Varma, *et.al*, 2016). Bioactive compounds are crucial (e.g., nutrients) and non-crucial (e.g., polyphenols, alkaloids, and many others) compounds that occur in nature, are part of the meals chain, and might have an effect on human fitness (Biesalski, *et.al*, 2009).

Dried materials are desired considering their long conservation time in comparison to clean samples. In case of fresh plant material extraction using natural solvents including ethanol or ethanol, is required to deactivate enzymes found inside the plant pattern. The extractive would possibly contain a full-size part of water; subsequently it may be

partitioned the usage of specific immiscible organic solvents (Jones, *et.al*, 2012). Drying is the most common technique to preserve the plant ingredient from enzymatic degradation, which includes hydrolysis of glucoside, etc. It have to be dried as speedy as viable within the open room under primitive conditions at ambient room temperature with air flow around the plant ingredients to steer clear of heat and moisture (Lalam, *et.al*, 2020). Plant material is dried at temperatures beneath 30°C to keep away from the decomposition of thermo labile compounds (Jones *et.al*, 2012).

Extraction of plant material:

Extraction is isolating the medicinally energetic mixture of many evidently lively compounds normally contained interior plant materials (tissues) using selective solvents via the same old system (Handa, *et.al*, 2008). It also can be described because the remedy of the plant material with solvent, whereby the medicinally active constituents are dissolved and maximum of the inert count remains undissolved. The motive of all extraction is to separate the soluble plant metabolites, leaving at the back of the insoluble cellular marc called residue (Sasidharan, *et.al*, 2018). The obtained product is incredibly complex mixture of metabolites, in liquid or semisolid state or (after casting off water) in dried powder form, and is intended for oral and/or outside uses. Extraction is based totally at the difference in solubility between the solute, different compounds within the matrix, and the solvent used to stabilize (Yolci Omeroglu, *et.al*,2019). The extraction of these lively compounds needs suitable extraction methods that keep in mind the plant components used as starting ingredient, the solvent used, extraction time, particle size and the stirring at some stage in extraction (Tušek, *et.al*, 2018). Extraction techniques encompass solvent extraction, distillation technique, urgent, and sublimation in step with the extraction principle. Solvent extraction is the maximum widely used technique (Lalam, *et.al*, 2020). In solvent extraction, the mass transfer of soluble elements to the solvent takes area in a concentration gradient. The mass switch price relies upon on the awareness of elements, till equilibrium is reached.

Classical or conventional techniques: Different methods used to employ on extraction procedure of plant extracts are:

1. **Maceration:** The process of maceration is carried out by soaking the plant ingredients (coarse or powered) in a closed stopper containing bottles in a solvent at room temperature for 2–3 days with periodic stirring to obtain plant extracts. An extractor (which is sealed) is used to avoid solvent dissipation at atmospheric pressure. The

process is done on purpose to soothe and smash the plant's cell walls to set free the soluble phytoconstituents. The mixture is then pressurized by filtration or decantation after a specific duration of time (Azwanida, *et.al*, 2015; Handa, *et.al*,2008). Maceration is a simple and widely used process. The extraction process works on principle of molecular diffusion which consumes a lot of time. Maceration ensures scattering of the concentrated solution accumulated around the surface of the particles and brings escort solvent to the particles surface for further extraction procedure (Zhang, *et.al*, 2010).

2. **Digestion:** The plant material to be extracted out is placed in a vessel with the pre-heated liquid to the appropriate temperatures (between 35 and 40°C), is maintained at half an hour to 24 hours with vigorously shaking the vessel. This process is mostly used for the herbal material or plant material that constitutes poorly soluble substances or polyphenolic compounds (Kamil Hussain, *et.al*, 2019). The temperature does not manipulate the active constituents of plant material, so there is greater efficacy in the use of menstruum.
3. **Infusion:** It is a simple chemical procedure used to extract plant ingredients that is volatile in nature and dissolves or set free its active constituents easily in organic solvents (Kamil Hussain, *et.al*, 2019). Infusion use the same principle as maceration involving soaking the plant ingredients in warm or cold water which is then permitted to steep in the liquid. The time for maceration is shorter in case of infusion. The liquid is segregated and concentrated under a vacuum using a rotary evaporator.
4. **Decoction:** It involves boiling the plant ingredients in water to obtain plant extracts. Heat is transmitted by convection and conduction and the preferred solvents will ascertain the type of compound extracted from the plant material (Azwanida, *et.al*, 2015). The sample is heated gently in a specified volume of water for 15 to 60 minutes then it is cooled, strained, filtered and a definite amount of water through the drug to obtain the desired volume.
5. **Percolation:** It is managed by passing the hot solvent along the plant material at a controlled and moderate rate till the extraction process is finished (before evaporation). The concentrated plant extracts are normally accumulated at the lowest of the vessel. To attain a great quantity of extract, successive percolations may be accomplished through refilling the percolator with fresh solvent and pooling all extracts together. This method is frequently used to extract energetic compounds inside the training of tinctures and fluid extracts. The system can be time-consuming and may require professional people (Kamil Hussain, *et.al*, 2019).

6. **Hot continuous extraction or Soxhlet extraction**: In soxhletation, finely ground pattern is kept in a porous bag or “thimble” crafted from a coarse filter paper or cellulose, set within the thimble chamber of the Soxhlet equipment. The first Soxhlet apparatus was developed in 1879 by Franz von Soxhlet (Soxhlet, *et.al*, 1879). Extraction solvents are heated in a round bottom flask, vaporized into the sample thimble, condensed in the condenser, and dripped back. Extraction solvents are boiled in a round bottom flask, evaporated into the sample thimble, liquidized within the condenser, and dripped off into the round bottom flask. When the liquid content material (containing the constituents) arrived at the siphon arm, the liquid containing constituents is emptied into the bottom flask once more, and the technique is continued (Azwanida, *et.al*, 2015). The hazards consist of no opportunity of stirring, and a big quantity of solvent is required. This technique is mistaken for thermo labile compounds as prolonged publicity (long extraction time) to warmness may additionally cause their degradation. It constitutes a professional classical method used to determine exceptional ingredients’ fat content material (Pandey, *et.al*, 2014; Yolci Omeroglu, *et.al*, 2019; Wang, *et.al*, 2006). Exposure to risky and flammable liquid natural solvents are the most noticed disadvantages on this method, and the excessive purity of extraction solvents wished can also add to the cost. Also, shaking or stirring can't be provided inside the Soxhlet tool to accelerate the manner (Wang, *et.al*, 2006). It requires a smaller amount of solvent as compared to maceration. Advantages of this technique include its easy operational mode, its applicability to a better temperature that increases the kinetics method, its low capital price, the lack of filtration, and the non-stop touch of the solvent and the pattern. It continues an extraordinarily high extraction temperature with warmth from the distillation flask (Yolci Omeroglu, *et.al*, 2019; Wang, *et.al*, 2006; De Castro, *et.al*, 1998).

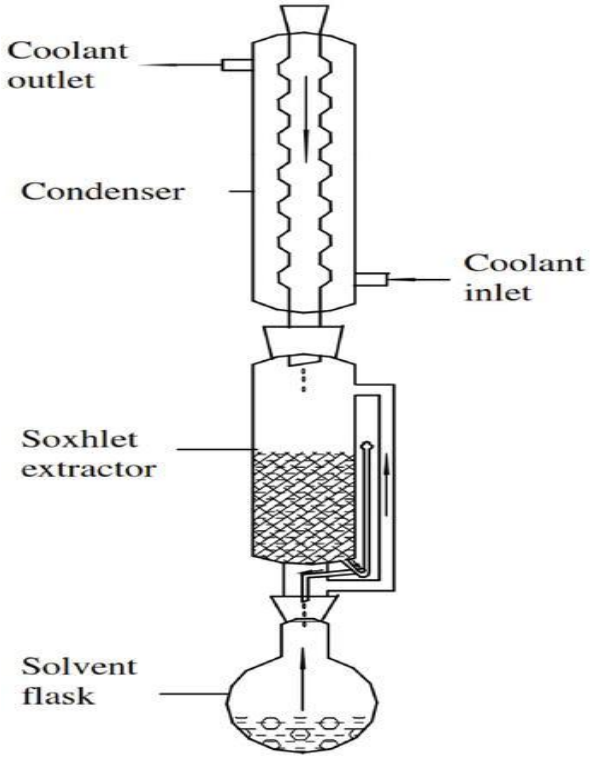


Figure 3: Soxhlet extraction apparatus.

Aims and objectives:

- Preparation of *Psoralea corylifolia* seed extract.
- Evaluation of antioxidant potential of *Psoralea corylifolia* seed extract.
- Investigation of the efficacy of *Psoralea corylifolia* seed extracts against non-small cell lung cancer A549 cells.

Material and Method:

Reagents and chemicals:

HiMedia, India; Merck and Sigma-Aldrich Co. (St. Louis, MO, USA) provided all the chemicals required for the study.

Plant Collection and extract preparation:

Seeds of *Psoralea corylifolia* was procured from Dr. AK Jain, BAMS, and Lucknow and authenticated by Dr. Mqbool Ahmad Khan, Deputy Director, CCRUM, Kursi Road, Basaha, and Lucknow.

Soxhlet Extraction:

Seeds of *Psoralea corylifolia* were crushed to the powder form. The powder was than subjected to conventional solvent based soxhlet extraction by using ethanol. The seed powder (20gm) was extracted with 250 ml of absolute ethanol for 7 hours at 70 degree temperature until the solvent become colorless. The solvent were then evaporated till dryness under reduced pressure and dried extract were then weighed and stored at -20 degree temperature for further use.

After evaporation, the extracted plant material of *Psoralea corylifolia* should be poured in the petri-plate and left for drying (1 or 2 days as per the requirement). When it is completely dried, scratch the extract using spatula or any pointed, flat equipment and was collected in an cell culture dish for weighing to observe the yield of the crude plant extract of *Psoralea corylifolia*. At the end of the extraction process, the weight of the extract of *Psoralea corylifolia* obtained was 3.0417 gm.

Preliminary Phytochemical Analysis:

Phytochemicals are the chemical components in plant life with distinct physiological action at the human frame**.** Alkaloids, flavonoids, phenolics, terpenoids, and essential oils are some of the essential bioactive phytochemicals**.** Active elements within the plant extract of *Psoralea corylifolia* have been diagnosed and detected by using performing chemical exams. Phytochemicals inclusive of tannins, phobatannins, saponins, terpenoids which includes flavonoids and alkaloids had been detected primarily based on general tests.

1. **Test for tannins:** About 0.1 g of dried powder plant sample was boiled in 4 mL of water in a test tube and then filtered. Few drops of 0.1% ferric chloride were added to observe brownish green or blue-black coloration indicative of the presence of tannins**.**

2. **Test for terpenoids (Salkowski test)**: The plant extract in a final volume of 3 mL was mixed with 1 mL of chloroform and 1 mL of conc. H_2SO_4 to observe the intense red-brown coloration indicative of the presence of terpenoids.

3. **Test for Phenolic compounds:** Compounds with a phenol group will form a blue, violet, purple, green, or red-brown color upon addition of aqueous ferric chloride. This reaction can be used as a test for phenol groups.

3ArOH + FeCl3 → Fe (OAr) 3 + 3HCl

- Mix several drops or a few crystals of compound to be tested in a beaker or in a 200 mm test tube.
- Add a few drops of 1% FeCl3.
- As a control, add a few drops of 1% FeCl3 to water in a second beaker. Also, mix aspirin in water to use as a control.
- Observe color change.

4. **Test for steroid:** The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (10 mL), then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids (Hossain, ***et.al***, 2013).

Determination of antioxidant activity:-

FRAP (Ferric Reducing Antioxidant Power) Assay:

Procedure:

- Take extract of different concentration in different test tubes (1 ml make-up).
- Add 2.5 ml PBS in all test tubes.
- Thoroughly mix it.
- Add 2.5 ml of 1% potassium ferricyanide solution in all test tubes.
- Vortex the sample prepared.
- Samples incubated at 50ºC for 20 minutes.
- Add 2.5 ml of 10% TCA in all test tubes.
- Centrifuges at 3000 RPM for 10 minutes.

- Collect the supernatant in separate test tubes.
- Add deionised water in all test tubes.
- Add 0.5 ml of ferric chloride, bluish color will be formed.
- Take measurement at 700 nm.

Samples having more concentration will show higher absorbance.

Determination of Anticancer activity:

The MTT staining assay was described by Mosmann with minor modifications**.** Anticancer activity of Psoralea corylifolia leaf extract on non-small lung A549 cells:

MTT cell viability assay: The MTT assay was used as a relative measure of cell viability.

- Cells were seeded at the density of $2X10^4$ cell/ml.
- Quadruple cell sample were grown in 96-well microtitre plates.
- After 24 hr, samples were exposed to different concentration of EO or standard compounds (0.39-200 microgram per ml) in a final volume of 100 microlitre of culture medium.
- Cells were incubated for 24h in a humidified atmosphere of 5% CO2 at 37ºC.
- At the end of incubation, each well received 10 microlitre of MTT (5mg/ml in phosphate-buffered saline (PBS)), and the plates were incubated for 2 h at 37ºC.
- The formazan crystals formed were solubilized in 100 microlitre DMSO after aspirating the medium.
- The extent of MTT reduction was measured spectrophotometrically at 595 nm using Fluorescence microscopes with LED illumination, and the cell survival was expressed as percentage over the vehicle. Experiments were conducted in triplicate. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50% (79.86±1.06). The IC50 values were determined with Graph Pad Prism5 computer program.

Morphological analysis:

Phase contrast microscope was used to examine the morphological alterations in A549 cells treated with *Psoralea corylifolia* extract. Briefly, non-small lung A549 cells ($5x10^3$) were cultured in a 96 well plate for 24 hr, followed by treatment of Psoralea corylifolia extract (50µg/ml, 100µg/ml, 200µg/ml) for 24 hr. Thereafter, phase contrast microscope was used to examine the morphology of A549 lung cancer cells.

Results:

1. Preliminary chemical screening: Analyzing the contents (Qualitative testing) present in the extract of *Psoralea corylifolia* plant through preliminary testing procedures.

A) Test for Tannins- After adding ferric chloride reagent to the filtered solution of the plant extract (0.25 g in 5 ml distilled water); blue-black, green or blue black precipitate was obtained hence it confirms the presence of tannins.

Figure 4: Presence for tannins in the *Psoralea* plant extract is confirmed.

B) Test for Terpenoids (Salkowski test)- A reddish brown coloration at the interface is obtained after mixing 5 ml extract in 2 ml chloroform and 3 ml of conc. H_2SO_4 hence confirms the presence of terpenoids.

Figure 5: Presence for terpenoids in the Psoralea plant extract is confirmed.

C) Test for Phenolic compounds: The plant extract was dissolved in 5 ml distilled water. After few drops of neutral 5% ferric chloride solution, green color was observed hence confirms the presence of phenolic compounds.

Figure 6: Presence for phenol in the *Psoralea* plant extract is confirmed.

D) Test for Steroids: There occurs formation of ring in between forming upper and lower compartments in the test tube. The upper compartment turns red and the lower compartment turns yellow with green fluorescence. It confirms the presence of steroids.

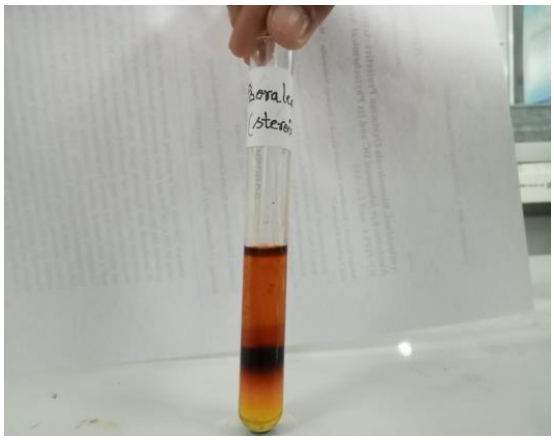
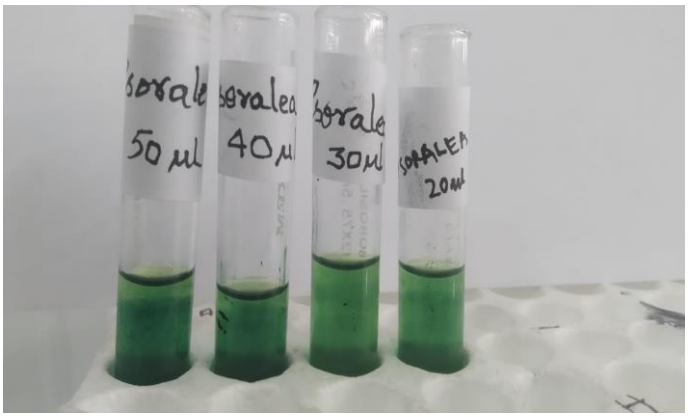


Figure 7: Presence for steroid in the *Psoralea* plant extract is confirmed.

2. Determination of Antioxidant activity:

FRAP (Ferric Reducing Antioxidant Power) Assay: The intensity of the blue color of sample prepared represents the concentration of the four different samples. The higher the concentration of the extract, the greater is the intensity of color of the sample and the lesser will be its anti-oxidant properties. As the concentration increases from 20 µl towards 50 µl, the antioxidant property increases accordingly.



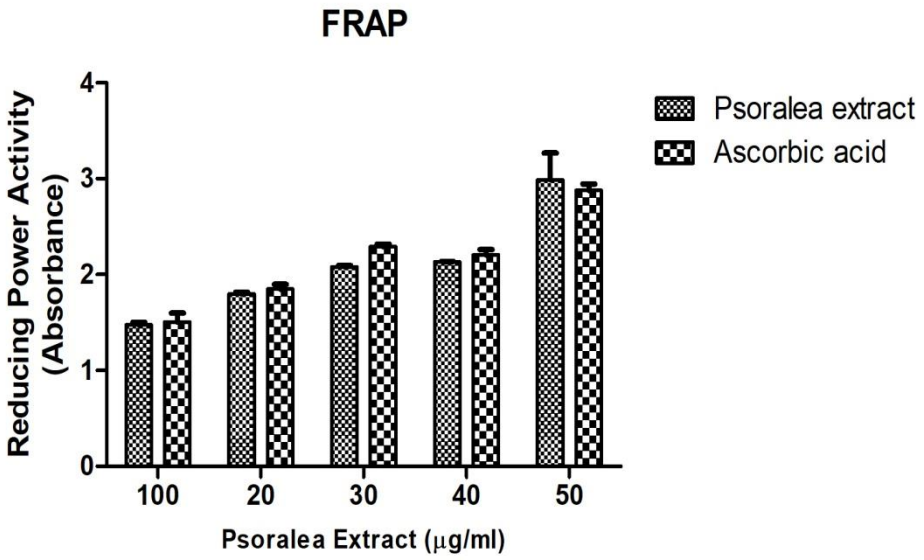
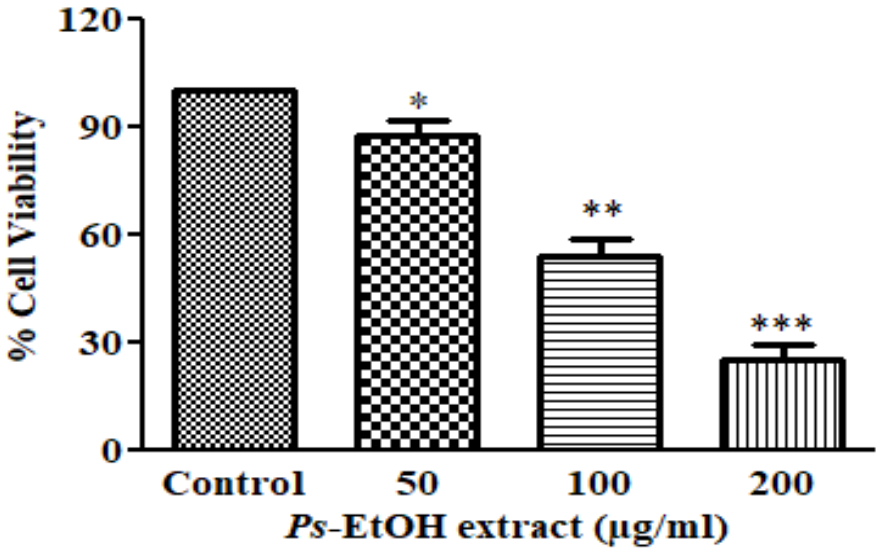


Figure 8: Antioxidant activity of *Psoralea* plant for four different concentrations of the extract.

The results of the absorbance (at 700 nm) of plant extract of *Psoralea corylifolia* obtained from four different concentrations of samples (20 µl, 30 µl, 40 µl and 50 µl) represented here as mean±SEM of three independent experiments. Mean±SEM of Psoralea extract at a concentration of 20 µl, 30 µl, 40 µl and 50 µl came out as 1.797±0.018, 2.083±0.012, 2.131±0.008 and 2.987±0.280 respectively.

Determination of anti-cancer activity:



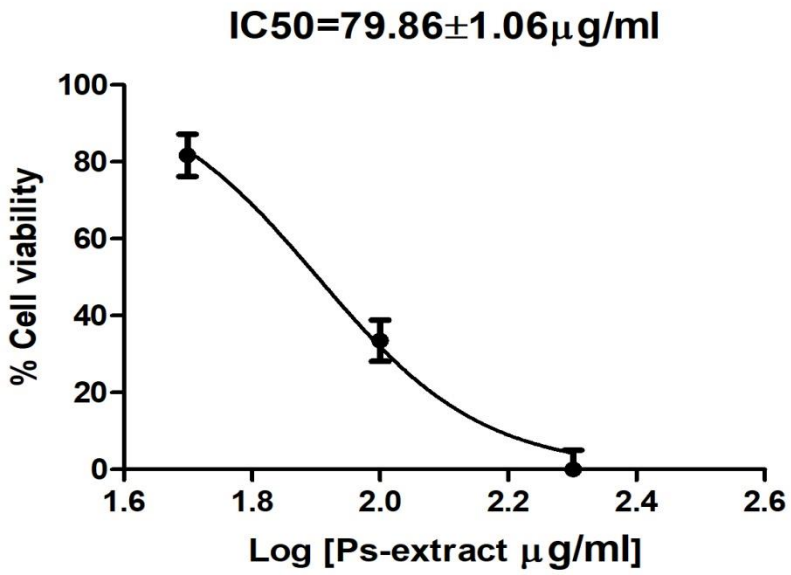


Figure 9: Percent cell viability of A549 lung cancer cells treated with different doses of Psoralea corylifolia (50µg/ml, 100µg/ml and 200µg/ml) for 24 hr assessed by MTT assay. Graph showed that Psoralea corylifolia exhibited an IC50 value (79.86±1.06µg/ml) at 24 hr, against A549 lung cancer cells. The results represented are the mean±SEM of three independent experiments performed in triplicate.

Psoralea extract induced morphological alterations in non-small lung A459 cells-

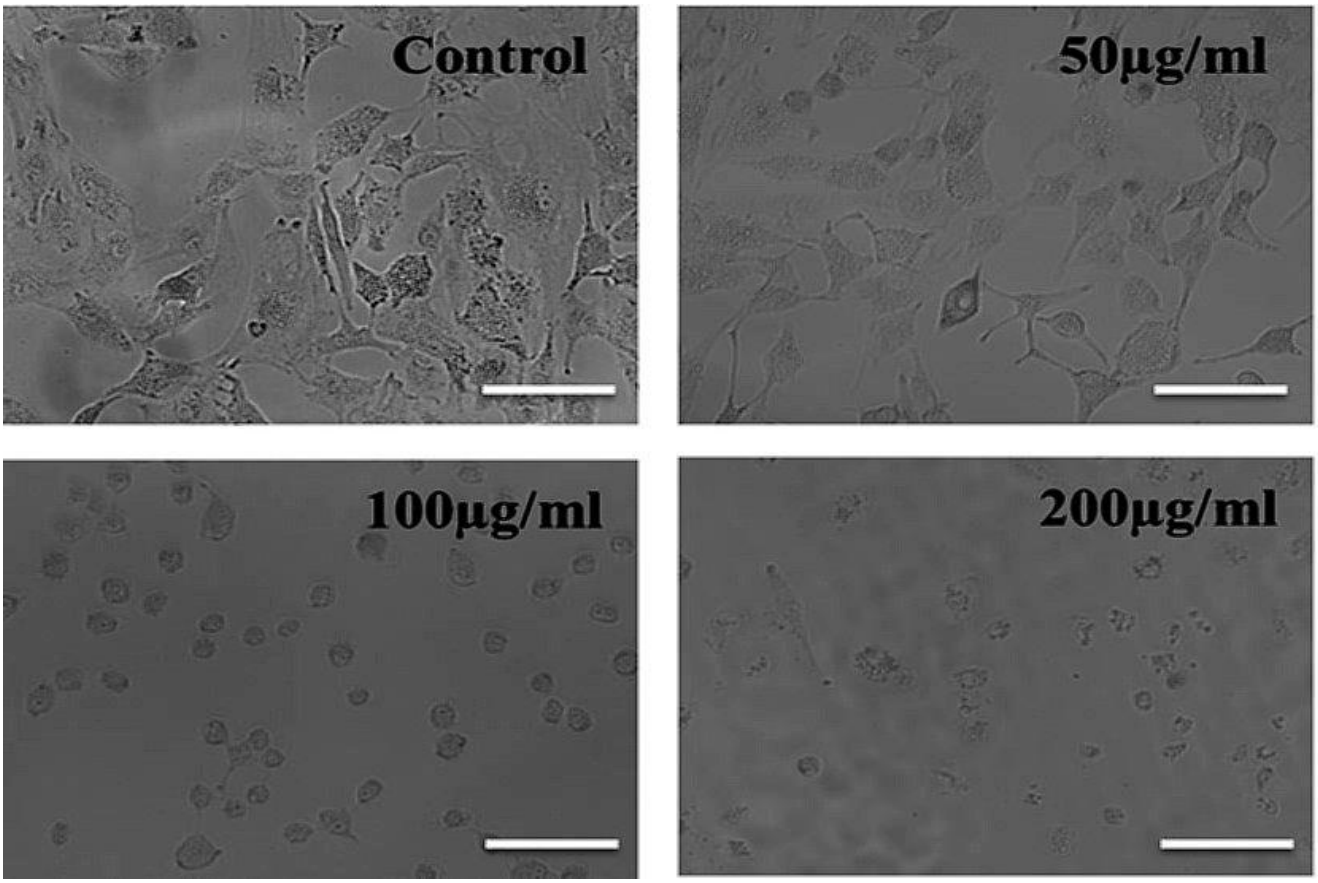


Figure10: The images of the untreated and treated lung cancer cell line (A549 cells) showed observable morphological changes under phase contrast microscope. The untreated cells revealed progressive cell growth with intact cell morphology under microscope. However, severe morphological alterations were noticed in *Psoralea corylifolia* extract treated A549 cells in a dose dependent manner (50µg/ml, 100µg/ml and 200µg/ml). Moreover, an increase in detachment and cytoplasmic shrinkage of cells were observed in *Psoralea corylifolia* extract lung cancer cells which resulted into greater number of floating cells. Thus, the results confirm that *Psoralea corylifolia* extract induce cytotoxicity in lung cancer cell line (A549 cells).

Discussion and Conclusion:

Psoralea corylifolia is usually observed as herb on the edge of a road and at waste locations at some point of India. The plant has been used since centuries in leucoderma, psoriasis, vitiligo, allergies, ulcers, kidney disorders, and as an aphrodisiac and an anti-inflammatory. It is reported to consist of vital oil, coumarins, alkaloids, flavonoids, and terpenoids. Concentrated fruit and seed extract may be determined in diverse natural preparations which can be in marketplace today. It is an important source of various forms of compounds with various chemical structures in addition to pharmacologic properties. Presence of such a wide range of chemicals shows that the plant may want to serve as a “lead” for the development of novel dealers having exact efficacy in diverse issues in the coming years. The performance and efficacy of the bioactive compounds gift inside the *Psoralea* species has been evaluated every so often. Now, due to the fact there's awareness complemented with scientific evidence concerning the medicinal advantages, their mass cultivation/propagation and extraction of bioactive compounds should additionally be the objective of similarly studies. The commercialization of pharmaceutical capsules containing *Psoralea* as a sole or a part of the elements shall convey relief to hundreds of thousands of people tormented by psoriasis, leprosy and vitiligo, in a natural way. To make sure sustained production and benefits of *Psoralea* products we should aim at its mass cultivation through conventional processes in addition to micropropagation. P. *corylifolia* truly showed that it is essentially crucial plant from ethnobotanical, pharmacological, and chemical point of view. P. *corylifolia* is a fortified supply of organic active com-pounds, which gives the plant with great value for its use in pharmaceuticals, health, and frame-care merchandise. Preliminary testing of *Psoralea corylifolia* seed extract indicates the presence of tannins, terpenoids, phenolic compounds and steroids. FRAP Assay proved that *Psoralea corylifolia* seed extract has significant levels of antioxidants when compared to the standard (Ascorbic acid). The MTT Assay performed on the Lung cancer A549 cells that had been treated with different concentrations of *Psoralea corylifolia* seed extract for 24 h showed reduced cell viability and proliferation successively per well as the Psoralea concentration increased. *Psoralea corylifolia* seed extract exhibited its IC50 on A549 cells at 79.86±1.06µg/ml for 24 h. This value showcases the efficiency of the extract in reducing the viability of Lung cancer A549 cells.

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