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ROLE OF *ALSTONIA SCHOLARIS LEAF* EXTRACT IN MODULATING THE CNS AND IN ITS ANTICANCER PROTECTION

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ABSTRACT

Due to its potential therapeutic properties in preventing and treating various disorders, the *Alstonia scholaris* leaf extract has been designated as an anticancer and central nervous system (CNS) modulating agent. The leaves of this Plant have a variety of activities related to their constituents, which was observed by Extraction using various types of Solvent according to their polarities, various literature and research articles reported the presence of most of the constituent, and here 2,3-secernate was selected as an active constituent in treatment of Cancer of various cell line (A549, HeLa, HepG2, HL60, and KB), respectively. In this activity, it was seen against Breast cell lines MCF-7 and T47D in Breast Carcinoma, and pyridine was used as a CNS-modulating agent in Swiss albino mice with a body weight of between 20 and 25 grams. The activity was measured by locomotor and rotarod activity. MTT-Assay was used to conduct in vitro cytotoxicity research on a 69-year-old female organism of the species Homo sapiens. Inactive mitochondria prevent even recently dead cells from efficiently cleaving MTT. Live cells create a dark blue formazan from MTT [3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrasodium bromide], a light-yellow substrate. Thus, calorimetrically assessing MTT cleavage may quantify the number of living cells. The percentage of growth inhibition also increased with increasing concentrations (in g/ml) of the ethanolic extract of *Alstonia scholaris*, which included the separated component C. This shows that the extract is very effective against cancer. Swiss albino mice weighing 20–25 grams were exposed to a chemical called pyridine, which altered their behavior. At doses of 50 and 100 milligrams, both the aqueous and ethanolic extracts showed CNS depressive effects, as measured by a decrease in locomotor activity counts and a delay in rotarod activity decrease.

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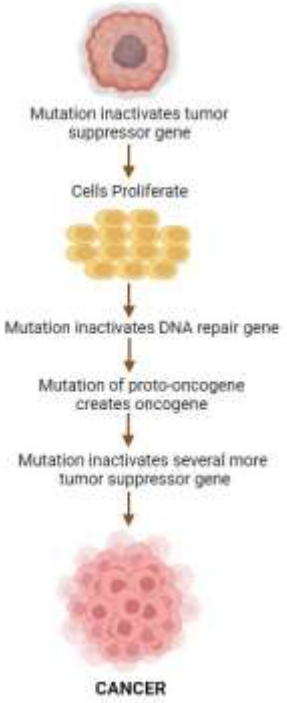
Keywords: A549 cells, HeLa cells, HepG2 cells, MTT, Alstonia Scholaris

INTRODUCTION

Cancer is caused by uncontrolled cell proliferation and metastasis. Multiple variables, such as heredity, the surrounding environment, and one's way of life, have been implicated in the development of cancer. Metastatic dissemination refers to the stage of metastasis in which cancer cells spread throughout the body through the lymphatic system and bloodstream. The illness may strike at any age, but the odds of getting it rise with time. This is because, as DNA ages, it becomes more susceptible to damage, which in turn raises the risk of illness. In 1775, a British specialist named Percivall Pott found that clears often suffered from scrotal growth. His findings led him to conclude that scrotal development was the root cause of cancer. The United States and the United Kingdom are only two of the Western nations where cancer now claims more lives than heart disease does.

MOLECULAR MECHANISM



A CASCADE OF MUTATIONS LEADS TO CANCER

Certain classes of chemical compounds are more likely to result in the development of mutations. For example, several aniline dye compounds have been linked to an increased risk of cancer. The carcinogens found in cigarette smoke are responsible for most deaths that occur in our modern civilization. Research conducted in laboratories on animals, studies on excessive alcohol use, and interviews with people who take certain drugs have all contributed to an increased

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understanding of synthetic chemicals' role in the development of illness.

SIGNS AND SYMPTOMS

There are typically three types of tumor complications:

Local symptoms include lumps or bumps that don't go away, a lack of drainage (when death is imminent), pain, or even an ulcer.

Jaundice is one symptom that might arise from the pressure of surrounding tissues.

Metastases are indicated by bloody coughs, enlarged livers, bone discomfort, fractured impacted bones, and neurological signs (Sharma, H., *et. al*., 2022).

FUTURE PROSPECTIVE:

In the late 1990s, cancer became an epidemic globally, making cancer research a particularly busy study subject. Oncological research aims to understand the mechanisms and therapeutics of cancer. Most treatments target cell surface receptors or damaged extracellular matrix surrounding the tumor: Immunotherapy, monoclonal antibody treatment, and homing devices. Radiation treatment is not the only cutting-edge cancer treatment method; radionuclide therapy is another option (Sharma, N.,*et. al.*, 2023). Anxiety, muscular tension, pain, sleeplessness, acute stress responses, panic attacks, and seizure disorders may all be treated with CNS depressants, which are given by psychotherapists. Some CNS depressants could be used as general analgesics if dosed more heavily Sharma, H., *et. al.*, 2022).

CENTRAL NERVOUS SYSTEM- A REVIEW

Most multicellular organisms, with the exception of sponges and radially symmetric species like jellyfish, rely on the central nervous system (CNS) to coordinate the movement of their many components. The brain, spinal cord, and retina are all located in this area, making up the bulk of the nervous system (Sharma, H., *et. al.*, 2023). It is protected by the vertebrae and the meninges and, together with the peripheral sensory system, plays a crucial role in behavioral regulation (Fontanarosa, 2000).

INTERACTION

Depressants of the central nervous system (CNS) should only be used in conjunction with other medications, such as stimulant solutions, and only under the guidance of a medical professional. Some naturally occurring remedies, such as Valerian and Kava, have the potential to dangerously amplify the effects of other substances that depress the central nervous system (CNS) (Iesa, M. A., *et. al.*, 2021). Consuming a combination of CNS depressants, such as alcohol and Valium, for example, is not encouraged in the same way. When taken together, substances that depress the central nervous system (CNS) have a propensity to amplify the effects of each other, which may result in an abnormally slow heart rate or even death (Sharma, H., *et. al.*, 2021).

TYPES

Liquor Alcoholic Beverages, Ethanol

Barbiturates

As effective as barbiturates are in relieving the symptoms of the diseases they are intended to treat, they are also highly addictive, physically dangerous, and pose the risk of fatal overdose. Benzodiazepines.

Miscellaneous

- Anticholinergic drugs include atropine, hyoscyamine, and scopolamine
- Diphenhydramine, doxylamine, promethazine, and other antihistamines Antipsychotics such haloperidol, chlorpromazine, and clozapine
- Dissociates, such as dextromethorphan, ketamine, phencyclidine, and nitrous oxide, and beta-blocking medications like propranolol and atenolol

- Muscle Relaxants (Carisoprodol, cyclobenzaprine) Zopiclone, zolpidem, and zaleplon are nonbenzodiazepines.

Aim and Objective

Alstonia scholaris has been used for a long time and in a wide variety of treatments because of the anti-inflammatory, antiulcer, fever, malarial fever, asthmatic, dyspepsia, antioxidant, hepatoprotective, anticancer, antifertility, and wound-healing effects of the plant's glycosides, alkaloids, flavonoids, steroids, and phenolic acid. The primary purpose of this research is to discover new medicines with anticancer and CNS-modifying properties in the aerial leaf component of *Alstonia scholaris* , as opposed to the bark, which has already been reported. It was decided to instead isolate the compounds responsible for the cytotoxic and CNS depressing characteristics of *Alstonia scholaris* leaves because of the poor yield and considerable harm to the plant from removing the bark.

Materials and methods

The Central Institute of Medicinal and Aromatic Plants in Lucknow, Uttar Pradesh, validated the validity of *Alstonia scholaris* leaves gathered in February 2017 in Varanasi, Uttar Pradesh, by the Council of Scientific and Industrial Research.

Studies of the Physicochemical Processes

The quality and purity of the powder made from the leaves of *Alstonia scholaris* was determined using a battery of physicochemical tests, including loss on drying, ash levels, and extractive value.

Total ash value

It was determined by placing 2 grams of powdered air-dried leaves into a tarred silica and burning it at a temperature lower than 450 degrees Celsius until the content was carbon-free, allowing the item to cool, and then reweighing it (Sharma, H., *et. al.*, 2022). If a carbon-free ash could not be obtained this way, the charred mass was purged with hot water, the residue was collected in an ashless filter paper, and the residue and filter paper were incinerated together until the ash was white or nearly so, the filtrate was added, the mixture was evaporated to dryness, and then it was ignited at a temperature no higher than 450 degrees Celsius. Its raw air-dried medicine's ash % was determined.

Ash that is insoluble in acid

The ash sample underwent a heating process within a gooch crucible, utilizing 25 ml of 2M HCl for a duration of five minutes. Following this, the resulting insoluble matter was gathered, rinsed with hot water, subjected to ignition, cooled within a desiccator, and ultimately measured for weight. The acid-insoluble ash proportion of the medication was determined based on its condition subsequent to the process of desiccation by exposure to air.

Ash that is water-soluble

The mixture was discarded after heating the ash for five minutes with water measuring 25 milliliters. It was concluded that insoluble materials should be collected. Crucible was composed of goo coupled with ash-free filter paper that had been washed in hot water and then burnt at temperatures lower than 450 degrees Celsius. The ash that is water-soluble is obtained by first determining the weight of the insoluble elements and then subtracting it from the weight of the ash. In order to calculate the %, this number was compared to the drug after it had been exposed to air and dried. Extractive values.

Water soluble extractives

After bringing fifty milliliters of water to a boil in a glass flask with a stopper, five grams of powdered air-dried leaves were then added to the flask. After giving it a vigorous shake and allowing it to settle for ten minutes, it was afterward cooled and filtered after being put through the filtering process. After transferring 5 ml of the filtrate to a tarred evaporating dish with a diameter of 7.5 cm, the solvent was evaporated in a water bath, the dish was allowed to dry for 30 minutes, and then the residue was weighed.

The dish was then allowed to dry for an additional 30 minutes before being weighed. A comparison was made between the crude air-dried medicine and the results of the computation in order to establish the proportion of water-soluble extractives present in the medication.

Alcohol soluble extractives

5 grams of air-dried plant material was macerated with 100 milliliters of alcohol for 18 hours in a covered vessel. After filtering, 25 milliliters of the filtrate were evaporated to dryness in a shallow dish with a tarred flat bottom. After that, the dish was dried at 105 degrees Celsius, and the plant material was weighed. The raw, air-dried plant material was used as a point of reference for determining the proportion of ethanol-soluble extractive that was present in the plant.

Extraction

During the extraction procedure, we utilized the gathered and cleaned powder of leaves from the *Alstonia scholaris* plant. In the Soxhlet apparatus, 200 grams of dried powder of leaves were uniformly packed, and a hot continuous extraction procedure was carried out for about twenty-six hours using a variety of solvents that increased in polarity from petroleum ether to chloroform to ethyl acetate to ethanol. After the processes described above, an aqueous extraction was accomplished by using a cold maceration technique. The extracts were filtered using Whatman filter paper while they were still hot in order to eliminate any contaminants that could have been present. The volume of the extracts was cut down to one-tenth its original size by a process called vacuum distillation.

After being transferred to a beaker with a capacity of one hundred millilitres, the concentrated extracts were then subjected to evaporation in a water bath. After that, to eliminate any moisture, the extracts were collected and stored in desiccators. Before being employed in future testing, the dried extracts were stored and labeled in an airtight container (Khandelwal and Pawar, 1996). These studies included phytochemical screening and pharmacological activity.

Preliminary phytochemical screening

The presence of phytoconstituents in *Alstonia scholaris* was determined by preliminary phytochemical screens of several extracts (Khandelwal, 2006).

Isolation

The leaves of *Alstonia scholaris* were subjected to air-drying and subsequently converted into a powder form weighing 8.5 kg. For each soaking at room temperature, the powder was steeped in 25 L 3, 95:5, v/v ethanol and water. The mixture was filtered. Following the vacuum concentration of the filtrate, the resulting residue was fractionated into six portions utilizing silica gel column chromatography (CC) with a benzene/ethyl acetate (90:10) gradient elution technique. The fraction was denoted using alphabetical characters A through F.

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Small and large Column chromatography of *Alstonia scholaris*

TLC, or thin-layer chromatography

Thin-layer chromatography was used to count components in each fraction. The testing was done on pre-coated plates. To test different extracts, benzene and ethyl acetate were mixed 90:10.

Pharmacognostic studies of leaves of *Alstonia scholaris*

Macroscopical evaluation the examination of a substance's taste, smell, and other sensory qualities (such as its touch and texture) is known as an "organoleptic evaluation." There should be pharmacopeial-quality samples and genuine examples of the item at hand for this purpose. Sense organs are useful for this since they consider these factors and, as a consequence, identify a discrete property of the item being examined. The purity and authenticity of the substance may then be determined from this. **(Mukherjee, 2008).**

Various fractions of *Alstonia scholaris*

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Microscopical evaluation

Preparation of specimen

Sectioned organ samples were submerged in FAA solution, which contains Farmalin (5 ml), Acetic acid (5 ml), and 70% Ethyl alcohol (90 ml). After 24 hours of sedimentation, Sass' 1940 timeline was followed to desiccate the specimens using a revised tert-Butyl fluid configuration. Paraffin wax, which softens at 58-60°C, was stretched to enter the samples until the TBA configuration reached super immersion. The specimens went into paraffin blocks.

Pharmacological Activity

Experimental animals

Swiss albino mice (20-25g) of both sexes were bought from IMS-BHU's experimental medicine and surgery center in Varanasi, U.P. for the present study. In sterile polypropylene cages with a 12-hour light/dark cycle, 27-29 degrees Celsius, and 56.5-58 percent relative humidity, the animals lived. You're free to eat and drink. A week before the study, the animals were habituated to the lab. The Centre for Experimental Medicine and Surgery approved the idea. ***(CEMS/IMS-BHU-129/07/017)***

Analysis of Acute Toxicity

Oral acute toxicity investigations are governed by regulations set by the Organization for Economic Co-operation and Development (OECD). It is an international group whose mission is to lessen the suffering of animals subjected to acute toxicity tests.

- ✓ The OECD generally adheres to the following types of guidelines:
- ✓ Fixed-dosage method (Guideline 420). (5 animals used)
- ✓ Class of Acute Toxicities (Guidelines 423). (3 animals used)
- ✓ Up-and-down technique (Guideline 425). (1animal used)

Requirement

Alcoholic and aqueous *Alstonia scholaris* leaf extracts.

Preparation of animals

The experiment used 20-25g mature male and female Swiss albino mice from the Institute. A standard mouse pellet diet (with or without water) was provided, and they were kept in conditions similar to those seen in most laboratories (about 60-70% humidity and 24-28 degrees Celsius). Specific testing plans, such as those included in this Annexure, illustrate the procedure to be followed for determining initial doses for first measurements. After keeping the animals fasted for a moderate period of time with just water, they were given extricates at a rate of 2000 milligrams per kilogram of body weight and monitored. If death was seen in more than half of the examined animals, the significance level should be decreased. Oral concentrations of 300 milligrams per kilogram of body weight (mg/kg b.w.) were monitored. To confirm the lethal dose, repeat the same experiment in which death was observed in a single organism.

Evaluation for in vitro cytotoxic activity

Live cells may produce a blue formazan by cleaving the yellow substrate 3-(4, 5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrasodium bromide (MTT). The utilization of this methodology necessitates the presence of functional mitochondria as cells that have undergone recent death exhibit minimal MTT breakdown. The quantity of MTT cleaved is directly proportional to the number of viable cells, as measured by colorimetric techniques. The experiment was carried out following the protocol established by Deshpande Laboratories located in Bhopal.

Apply a blue hue to the protein structures and related components. To generate a variety of test concentrations, the chemical substances were initially dissolved in DMSO and subsequently underwent serial dilution using a complete medium. A consistent DMSO concentration of

0.1% was upheld in all samples. The experiment involved seeding well-maintained cell lines in 96-well plates, followed by treatment with different dosages of the test substances. The plates were then incubated at a temperature of 37°C and a CO2 concentration of 5% for a duration of 96 hours. Following a 4-hour incubation period with MTT reagent, the cells produced a dark blue formazan product within the wells. This product was subsequently dissolved in DMSO and analyzed at a wavelength of 550nm within a biosafety cabinet. The inhibitory concentration 50 (IC50) values and corresponding concentrations were presented as percentages of inhibition.

Evaluation for *in vivo* CNS activity

Swiss albino mice of either sex (20-25 g) was randomized into six groups of six animals, tagged to enable individual identification, and acclimated to laboratory settings for at least 5 days before dosing.

Group 1- Normal (treated with normal saline (10ml/kg, i.p.))

Group 2- Standard (treated with Chlorpromazine hydrochloride 3mg/kg i.p.)

Group 3- Test A (treated with 50 mg/kg of aqueous extract of *Alstonia scholaris*)

Group 4- Test B (treated with 100 mg/kg of aqueous extract of *Alstonia scholaris*)

Group 5- Test C (treated with 50 mg/kg of ethanolic extract of *Alstonia scholaris*)

Group 6- Test D (treated with 100 mg/kg of ethanolic extract of *Alstonia scholaris*)

Procedure for *in vivo* CNS activity

Locomotor activity

The mice should be placed in the exercise cage and the equipment turned on for a period of 10 minutes. All of the animals' resting activity levels have been documented. After injecting Chlorpromazine and the test medication (a plant extract at 50 and 100 milligrams per kilogram, respectively), the mice were retested for activity levels for further 10 minutes.

Rotarod apparatus

Start the Rotarod and adjust the speed to 20–25 revolutions per minute. Carefully set each animal on the turning rod. Make a note of when the mice fall off the spinning rod. According to a recent study (Kulkarni, 2009),

Analytical Statistics

For each group, we determined a mean and standard deviation. Statistical significance was determined using One-way ANOVA and Dennett's test at the P0.01 and P0.05 levels, respectively. There was a control group for each experimental group.

Compounds and chemical components and reagents

All of the solvents and chemicals were of laboratory-grade purity. Merck in Mumbai was able to provide silica gel in the 200-400 mesh range. In Mumbai, we got our chloroform from a place called Himedia. The Chinese company Chang Shuyang Yuan Chemicals supplied the ethanol. Additional chemicals and solvents were purchased from a nearby shop.

Result

Alstonia scholaris (Apocynaceae) was chosen after discussion with locals and research. The sample was first obtained in the city of Varanasi in the month of January 2017, and then transported to CSIR-CIMAP in Lucknow, Uttar Pradesh, for further examination.

After drying, coarsely crushing, and cold macerating the leaves of *Alstonia scholaris* , we tested the effects of increasing the polarity of the solvents used. The obtained yields for extracts prepared using different solvents, namely petroleum ether, chloroform, ethyl acetate, ethanol, and water, were found to range from 1.5% w/w to 3.6% w/w. *Alstonia scholaris* water extract was found to have a higher percentage yield than the alcoholic extract.

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Numerical data acquired from physicochemical research was essential in the selection and authentication of *Alstonia scholaris* for further exploration of their traditional uses.

It was determined that at least 5.26 percent of the powdered medicine's weight was lost during the drying process. The ash concentration of a crude medicine may reveal its inorganic or earthy composition and the presence of any contaminants. Ash, acid-insoluble ash, and water-soluble ash concentrations were all measured for this investigation. Overall ash and acid-insoluble ash percentages can't be more than 8.67% and 0.8%, respectively. The highest percentage of water-soluble ash found was 1.7% w/w. A minimum extraction value of 8% w/w in water and 16% w/w in alcohol was established. The results showed that plant parts are more soluble in alcohol and less soluble in water. During the preliminary phytochemical screening of several extracts using a suitable reagent, petroleum ether yielded a positive result for flavonoids, alkaloids, and triterpenoids.

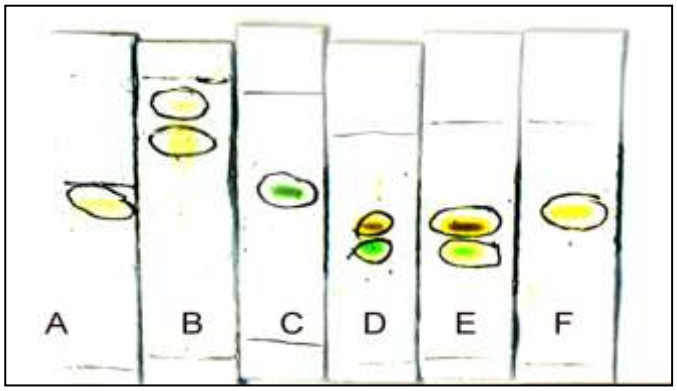
- A chemical study of chloroform revealed that it contains alkaloids.
- Alkaloids and triterpenoids performed well in ethanol tests.
- Positive findings were found for alkaloids, flavonoids, and saponins in the water.

Thin Layer Chromatography (TLC)-

T Table 2 displays the results of thin-layer chromatography used to determine the concentrations of individual components within the different fractions.

Table no.2

S. No.	Fraction	Spot	Rf value
1	A	One	0.7
2	B	Two	0.52, 0.73
3	C	One	0.62
4	D	Two	0.5 , 0.69
5	E	Two	0.48 , 0.74
6	F	One	0.5



TLC of various fractions

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Macroscopic features of the leaf

Colour - Green

Odour - Aromatic

Taste - Bitter

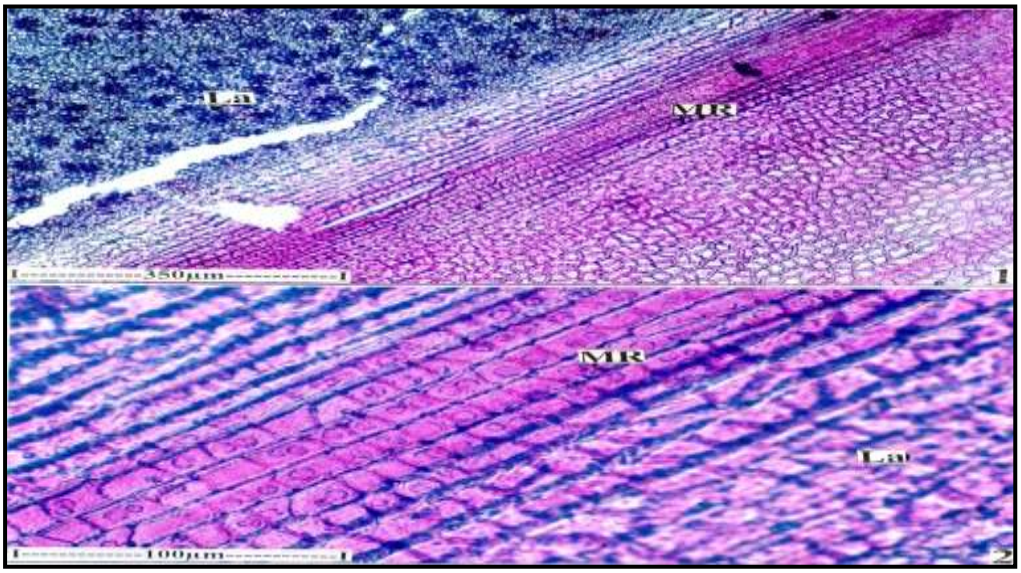
Leaves- 4-7 in a whorl

Size - 5-15cm length 2-7cm wide

Apex- Usually rounded

Results for Powder microscopic studies of the leaf

Small pieces of leaf epidermis may be found in the powdered leaves. Veins may be seen on the epidermal fragments. Near veins, the epidermal cells are square and arranged in parallel rows. The cell membrane is robust and undulating. The papillate projections of the basal membrane of the cell may be seen as a rounded structure inside each cell. The cells in the vein's lateral zone are noticeably longer, thicker-walled, and undulating. Also included are bits of the top epidermis. As may be seen from the outside, the higher (adaxial) epidermal cells have a polyhedral shape. Anticlinal walls are strong, uniform, and pitted in a straightforward fashion. Abaxial epidermal peelings are also seen in the dust. The abaxial epidermal layer is staminiferous because the stomata have contracted and are no longer easily discernible. Small in size, with paper-thin walls and a curvy form, epidermal cells make up the outermost layer of the skin.



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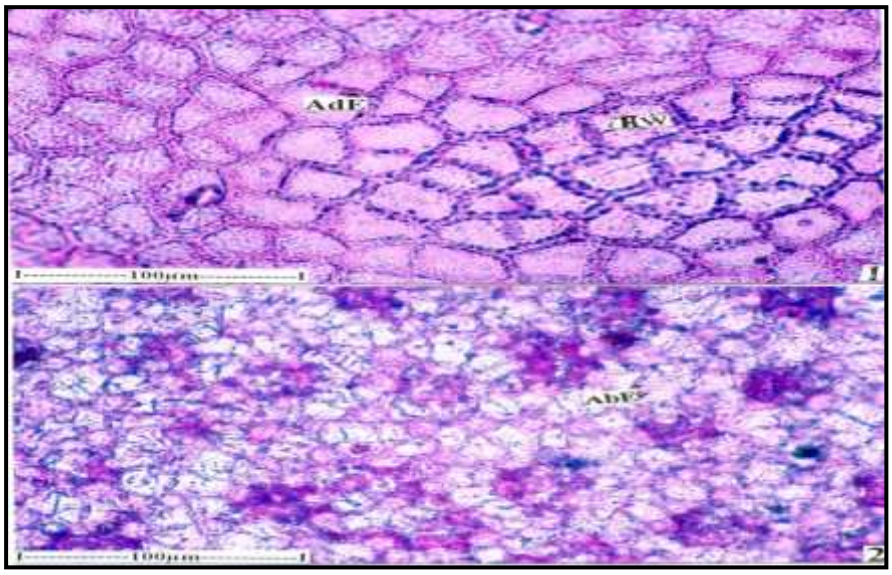
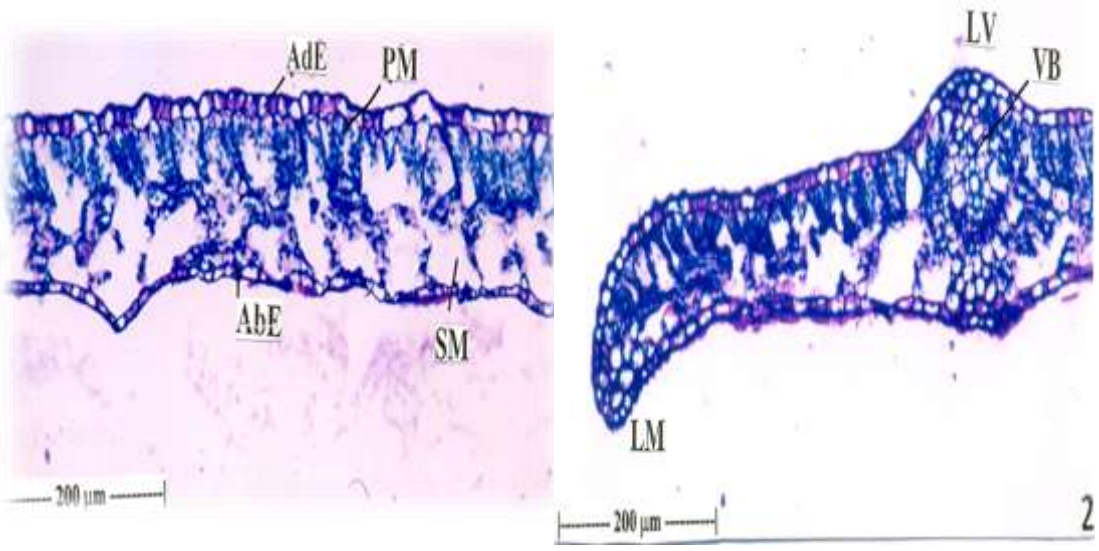


TS of lamina



TS of midrib- entire view

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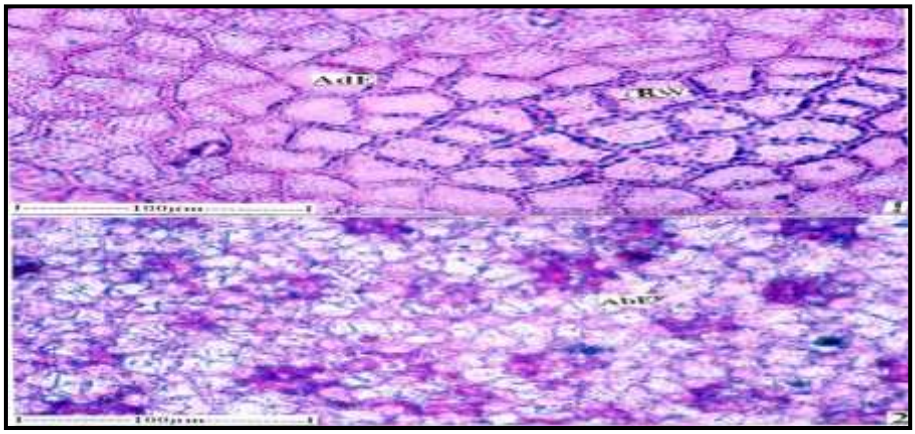

Venation system of the lamina

Pharmacological study

Result of acute toxicity

In order to calculate the LD50 value, an acute toxicity research was performed using OECD guidelines on both the aqueous and alcoholic extracts of the leaves of *Alstonia scholaris* . According to the data, extracts fall within GHS category 4 (pH 300-2000). The LD50 was calculated to be 500 mg/kg, and the ED50 was calculated to be 50 mg/kg based on these data. Table 3 shows the outcomes.

Table no. 3

Group	Animals Counted	What Is the Dosage (mg/kg)?	Result
1.	Animals: 3	2000	3 deaths
2.	Animals: 3	300	1 death
3.	Animals: 3	300	1 death

Evaluation for in *vitro* cytotoxic activity

The in vitro cytotoxic activity was tested on an ethanolic extract of *Alstonia scholaris* produced from fraction C using a benzene: ethyl acetate (90:10) solvent in column chromatography. Human breast cancer cell line T47D (Homo sapiens, Human) was utilized in an MTT experiment to measure cytotoxic activity in vitro.

In vitro cytotoxic activity was measured using an ethanolic extract of *Alstonia scholaris* produced by column chromatography using a benzene: ethyl acetate (90:10) solvent. Breast cell line MCF 7 (species: Homo sapiens) was used in an Quantity of Fraction c extracted from *Alstonia scholaris* ethanolic extract (in g/ml) correlated with % of growth inhibition.

Dose response curves for compounds against T47D.

On X axis – concentration in microgram/ml

On Y axis- % growth inhibition

MTT experiment to determine cytotoxic activity in vitro. Growth inhibition increased with Fraction C concentration (mg/ml) of *Alstonia scholaris* ethanolic extract.

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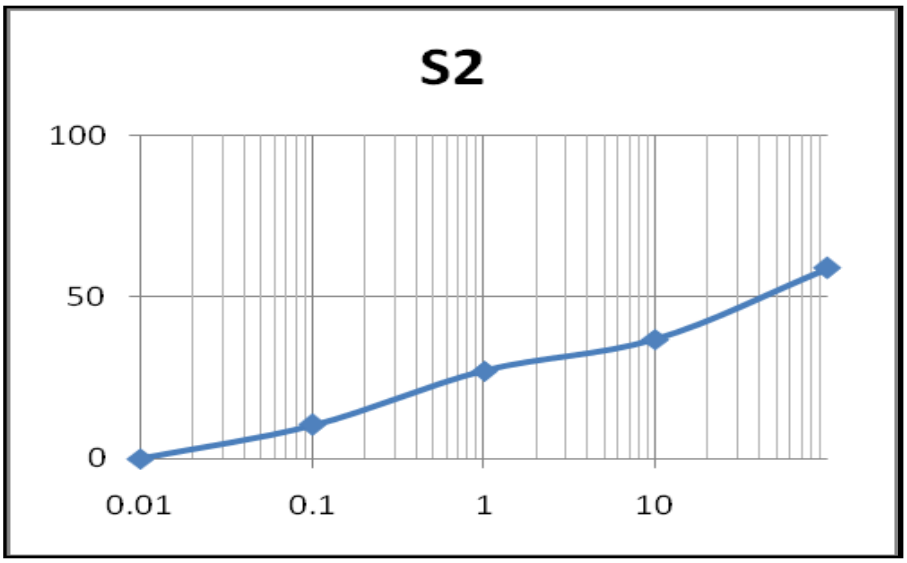

On Y axis- %

Growth inhibition On X axis – concentration in microgram/ml

Response curves for compounds against MCF 7

Result of In Vivo CNS activity

Alstonia scholaris extracts substantially reduced the activity of swiss albino mice in a maze test compared to the control group. These values resulted in a reduction in locomotor activity of 224, 222.5, 204.16, and 196 for aqueous extracts and ethanolic plant extracts, respectively, at 50 and 100 mg/kg. Finding that 100 mg/kg of ethanolic extracts generated the greatest reduction in locomotor activity compared to the 40.66 mg/kg reduction achieved by the standard medication chlorpromazine. In table 4, we see the outcomes of locomotor activity.

Table no. 4:

Groups	Treatment	Locomotor activityfor 10min	
		Before	After
I	Control (Normal saline)	236.5±3.87	236.66±4.12
II	Chlorpromazine(3mg/kg)i.p	236.66±3.79	*40.66*±1.35**
III	Aqueous Extract 50mg/kg	*234.16*±4.55	*224*±3.11*
IV	Aqueous extract 100mg/kg	*238.32*±4.42	*222.5*±3.18*
V	Ethanolic extract 50mg/kg	*234.67*±3.80	*204.16*±2.1**
VI	Ethanolic extract 100mg/kg	*235*±4.93	*196*±2.06**

(Mean SEM, n=6. (Statistical significance between the experimental and control groups was determined using a one-way analysis of variance and Dunnett's multiple compression test; *, ** indicate P0.05 and P0.01, respectively)

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Table no.5

Group	Treatment	Rotarod activity after treatment
I	Control (Normal saline)	676±16.35
II	Diazepam (3mg/kg)i.p	278.83±2.88**
III	Aqueous extract (50mg/kg)	620.16±16.95*
IV	Aqueous extract (100mg/kg)	615±16.70*
V	Ethanol extract (50mg/kg)	589±14.02*
VI	Ethanol extract (100mg/kg)	577±14.5*

Statistical significance was determined in contrast to the control group using one-way analysis of variance and Dunnett's multiple compression test (mean standard error of the mean; n=6).

DISCUSSION

Alstonia scholaris is a medicinal herb with several uses. Preliminary phytochemical screening and physicochemical tests suggest that this plant may be useful for treating a number of medical conditions. Both macroscopic (using a microscope) and microscopic (using a microscope and T.S.) pharmacognostic tests were conducted. Collenchyma, palishade, xylem, phloem, stomata, and the epidermal layer were all visible in microscopic investigations (T.S.). Epidermal peddling, round spots inside individual cells, and Abaxial epidermal peelings predominated in powder microscopy.

The dried leaves of *Alstonia scholaris* were subjected to a hot, continuous extraction process for approximately 26 hours. Petroleum ether, chloroform, ethyl acetate, and ethanol were used for extraction. We used a cold maceration procedure to remove water from the plant leaves. *Alstonia scholaris* has a 1.8%, 2.2%, 1.5%, 3.2%, and 3.6% yield using different solvents. Extracts from these plants were found to include wax, flavonoid, saponin, steroid terpenoids, and alkaloids in chemical analysis. TLC analysis of a range of extracts in a variety of solvent systems revealed a wide range of constituents in each sample. These components have Rf values of 0.76, 0.35 (Pet. ether), 0.48, 0.9 (chloroform), 0.14, 0.33, 0.77 (ethyl acetate), 0.5 (ethanol), 0.43 (water), 0.6, and 0.53 respectively.

Picrinin, a monoterpenoide indole alkaloid, has been found in *Alstonia scholaris* leaves (Xiang et al., 2008). Planta Medica reported in 1976 that picrinin has a CNS depressive effect. *Alstonia scholaris* extract substantially reduces locomotor and rota rod activity in Swiss albino mice, according to a research using an ethanolic and aqueous extract to examine its CNS depressive action. Picrinin inhibited locomotor and rota rod activity in ethanolic extracts most at 100 mg/kg. GABA is the central nervous system's main inhibitory neurotransmitter. *Alstonia scholaris* may directly activate GABA receptors or potentiate GABAergic inhibition in the CNS through membrane hyperpolarization, decreasing the firing rate of key brain neurons (Verma, 2010). *Alstonia* sp. phytochemical components have been studied in depth. Formulated with raw *Alstonia scholaris* leaves we were able to extract two different Alstonic acids A and B were extracted from 2,3-secofernane triterpenoids. The triterpenoids 1 and 2 are the first of their kind to be reported. If present, they might serve as useful indicators for chemotaxonomic analyses (Fei wang et al., 2009).

Alstonia scholaris extract was prepared by evaporating a solution containing dried, powdered leaves steeped in a mixture of ethanol and water (95:5) (25:3). The ethanolic extract yielded 8.82%. Use a standard procedure developed for packing tiny columns to pack big

ones. Get a tiny glass column and prepack it with extract and silica (200-400) at a ratio of 1:30 (grams) of extract to silica. Then, the mobile phase of benzene and ethyl acetate (90:10) was put onto the column. Determined the total number of bands and compiled the corresponding A, B, C, D, E, and F fractions.

Different fractions took 32, 25, 46, 23, 28, and 37 minutes to elute. The extract and silica (200-400 gram) were then used to stuff the massive column. The number of bands and their percentage were tallied. Each fraction's elution time ranged from 2.5 hours to 45 minutes to 3.49 hours to 2.15 hours to 1.2 hours to 4.2 hours. There was a search for terpenes in the fractions. Terpens were absent in the A, B, and F fractions, but they were present in the C, D, and E fractions. The fractions 1, 2, 1, 2, 2, and 1 were the most common. The cytotoxic investigation on MTT test was performed on fraction C since it contained 2,3- secofernane tritrpenoids and exhibited a single spot. In order to produce a dark blue formazan result, live cells cleave a light yellow substrate called MTT [(3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetra sodium bromide] to reveal its active ingredient, tetrazolium bromide. Cleavage of MTT is dependent on functional mitochondria; recently deceased cells are unable to cleave any MTT at all. Therefore, the number of live cells may be determined by measuring the quantity of MTT cleaved using colorimetric techniques.

Breast cell line T47D (human) and breast cell line MCF 7 (human) were used in an MTT experiment to determine cytotoxic activity in vitro. The concentration (g/ml) of the ethanolic extract of *Alstonia scholaris* 's separated Fraction C enhanced the percentage of growth inhibition.

SUMMARY AND CONCLUSION

In terms of pharmacology, the *Alstonia scholaris* plant is quite versatile. As a medication, it has long been a staple of many different traditional medical practices. It has been used for ages as an astringent, demulcent, appetizer, arthritis cure, tonic, aphrodisiac, and diarrhea remedy. Recent studies have shown that it has other applications, including antibacterial, antifertility, anti-inflammatory, and antiemetic effects.

Based on physicochemical studies and preliminary phytochemical screening, it appears that this plant may have a significant effect against cancer and CNS modulating activity, and a review of the available literature suggests that much more could be learned about its leaf. Our long-term goal is to define the chemical makeup of the purified ethanolic extract of *Alstonia scholaris* leaf material.

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