



Cytotoxic Effect of Aqueous and Ethanolic Bark Extracts of *Alstonia Scholaris* against Cervical Cancer Cell Line

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ABSTRACT

Alstonia scholaris, a tropical evergreen tree belonging to the family Apocynaceae has traditionally been referred to have anti-cancer activity, but it has not been explored so far. Therefore, the study is aimed to investigate the cytotoxic effect of aqueous and ethanolic bark extracts of *Alstonia scholaris* against cervical cancer cell line (HeLa Cell line). The bark part of *Alstonia scholaris* was collected, dried, powdered and made to a coarse powder. Maceration was carried out for the bark of *Alstonia Scholaris* using two different solvents like ethanol and aqueous solutions. Both the extracts were collected, evaporated to dryness. The extracts were then investigated for the phytochemical screening and cytotoxic effect by MTT Assay using cervical cancer cell line (HeLa cell line). The phytochemical screening of both the extracts showed the presence of various phytoconstituents such as alkaloids, flavonoids, glycosides, gums and mucilage. The IC₅₀ value of *Alstonia scholaris* of aqueous extract was found to be 13.38 μg/ml, whereas the IC₅₀ value of ethanol extract was found to be 14.21 μg/ml. According to the results obtained, it has been proved that the aqueous and ethanolic extracts of *Alstonia Scholaris* bark showed the presence of significant phytoconstituents and also showed significant cytotoxic effect (MTT Assay) against cervical cancer.

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INTRODUCTION

Globally, cancer is one of the common deadly illness that spreads through the bloodstream in the human body (WHO, 2018). It involves abnormal cell growth

and travels to other organs also (WHO, 2014). Cancer is not one disease but a group of more than 100 distinct disorders (KunduSen et al., 2011).

Cancer is differentiated into several types. In women, next to breast cancer, cervical cancer occurs frequently among them (Cragg and Newman, 2005) and in 2018, about 570,000 new cases has been reported. Developing countries and underdeveloped countries account for 90% of death approximately due to cervical cancer, and this could be reduced by comprehensive approaches like prevention, early diagnosis, effective screening and treatment programmes (Jenson and Lancaster, 1990).

Nowadays, cervical cancer vaccines are available that are effective against human papillomavirus, which significantly reduces the risk of cervical cancer. Cervical cancer occurs in the cervix part of

the female, which is a cylinder-shaped neck structure that connects the vagina and uterus (Ferlay and Globocan, 2000) , (Colombo et al., 2012). Cancer which occurs at squamous cells in the cervix is known as squamous cell carcinoma, whereas cancer which occurs at glandular cells is known as adenocarcinoma (Australian Institute of Health and Welfare (AIHW), 2017) .

Alstonia Scholaris (dita bark or devil tree) is a tropical evergreen tree belonging to the family Apocynaceae. It contains hard bark that is greyish in colour and milky sap that contains alkaloids. This plant belongs to the nativity of Indian sub-continent, Sri Lanka, Pakistan, Nepal, Thailand, Burma, South-east Asia and WestBengal (Christophe, 2006). The plant grows up to 17-20m in height and 110cm in diameter. The plant is used in Ayurveda, Unani, Siddha type of alternative system of medicine. It is an important medicinal plant in curing various human diseases (Khare, 2007).

In *A.Scholaris*, the bark has a high quantity of mucilage, gums, alkaloids, glycosides, compared to that of stem and leaf. Phytochemical reviews in bark and leaves revealed the presence of major phytoconstituents such as alkaloids, Terpenoids, flavonoids, phenolic compounds, saponins, tannins and glycosides (Vaidyanatha et al., 2011).

The compounds isolated from the leaves of *Alstonia Scholaris* are cycloeucalenol, cycloartenol, lupeol, lupeol acetate and botulin. Further studies have been performed in the flowers to isolate the compounds such as mixtures of α -amyrin acetate, β -amyrin acetate and lupeol acetate, β -amyrin fatty acid esters, lupeol fatty acid esters (Dey et al., 2011).

Alstonia Scholaris contains alkaloids such as 19-episclolaricine, Some of the alkaloids present in *A.Scholaris* are 19-episclolaricine, echitanine, ditamine, strictamine, N β -methylscholaricine, N α -Methylburnamine and Vallesamine N β -Oxide, picrinine, nareline, alschomine, angustobine (Wang et al., 2016; Kaushik et al., 2011).

A. Scholaris has many medicinal uses like antimicrobial, anti-amoebic, antidiarrheal, anti-hypertensive, anti-malarial, febrifuge, stimulant, hepatoprotective, immunomodulatory, anticancer, anti-asthmatic, antioxidant, analgesic, anti-inflammatory, anti-fertility, anti-diabetic etc. It is also used in the treatment of fevers, chronic diarrhoea, dysentery, ulcers, rheumatic pains, cancer, malarial (Nadkarni and Nadkarni, 1976; Atta-Ur-Rahman et al., 1985).

Therefore, the present study involves the investigation of the Cytotoxic Effects of Aqueous and Ethano-

lic Bark Extracts of *Alstonia Scholaris* against Cervical Cancer Cell Line.

MATERIALS AND METHODS

Collection of plant and identification

The bark of the plant *Alstonia scholaris* was used for this study Figure 1 . It was collected from Tirupathi, Andra Pradesh and the taxonomical identification of bark was confirmed by Dr K. Madhava Chetty, plant taxonomist, Tirupathi, Andra Pradesh, India.

The plant collected was then washed under a clean running tap water, air-dried for 2-3 weeks and then regrind into coarse powder and used for further studies.



Figure 1: *Alstonia Scholaris* (Bark)

Reagents and materials

The chemicals and solvents-Ethanol used for the extraction were purchased from southern India Scientific Corporation, Chennai, India.

Extraction method

Maceration technique is used for the extraction process. The plant materials were ground and made into a coarse powder. About 100gms of powder was taken in a two stoppered container individually. To one container, 1000ml of distilled water is added, and to another container, 1000ml of ethanol was added.

Then, they were allowed to stand at room temperature with frequent agitation for a minimum of 3 days.

The mixture after that is strained, the marc which is the damp solids material is pressed, and the two liquids are made clear either by filtration or decantation and then concentrated by evaporating the liquid contents and dried to get the crude residue.

Phytochemical analysis

The following methods were applied to analyze the presence of phytochemicals present in Ethanol

Table 1: Phytochemical analysis of *Alstonia Scholaris*

Phytochemicals	Aqueous Extract	Ethanol Extract
Alkaloids	P	P
Glycosides	P	P
Flavonoids	P	P
Steroids and sterols	A	A
Oils and Fats	P	P
Gums and mucilage	P	P
Vitamins	P	A

P-Present A- Absent

Table 2: *In-Vitro* cytotoxicity effect of aqueous extract and ethanol extract of *Alstonia Scholaris* against HeLa cell line

Sample Concentration	HeLa cell viability %	
	Aqueous extract	Ethanol extract
0	100	100
1.5625	90.7±0.2	93.7±0.2
3.125	79.4±0.2	76.5±0.2
6.25	73.4±0.6	73.4±0.2
12.5	58.1±0.1	61.8±0.3
25	47.4±0.04	51.34±0.3
50	38.3±0.1	41.1±0.3
100	27.3±0.3	30.34±0.5
200	16.7±0.1	18.94±0.1
IC50	13.38 µg/ml	14.21 µg/ml

and Aqueous extracts of bark of *A.Scholaris* (Reddy, 2016; Dhruvi et al., 2016).

Alkaloids

Dragendorff's test

1ml of distilled water was added to 2mg of an aqueous and ethanol-soluble fraction of the extract, followed by the addition of 2 M (HCl) Hydrochloric acid until initiation of acid reaction. After that, Dragendorff's Reagent of 1ml was added to the mixture and resulted in the formation of an orange precipitate.

Hager's test

2mg aqueous and ethanol extracts were added to a few drops of Hager's Reagent. It produces a yellow precipitate.

Wagner's test

2 mg of the aqueous and ethanol-soluble fraction was made acidic with hydrochloric acid 1.5% v/v. Then few drops of Wagner's Reagent was added, and it forms a brown precipitate.

Mayer's test

2mg of aqueous and ethanol extract was added to a few drops of Mayer's Reagent that results in the for-

mation of white or pale yellow precipitate.

Flavonoids

To 1mg of aqueous and ethanol extract, few drops of sodium hydroxide solution and dilute Hydrochloric acid was added.

First, the solution becomes yellow, which then turned to a colourless solution.

Phenols and tannins

To the extracts, add a few drops of FeCl₃ solution. The solution turns into a blue or bluish-green colour.

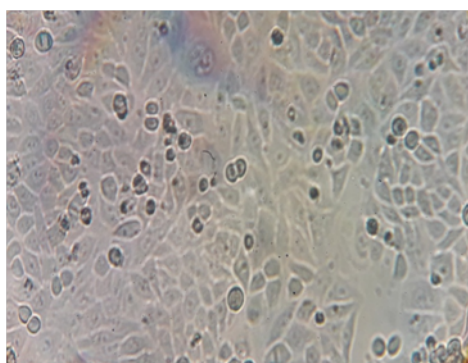
Proteins

5mg of ethanol and aqueous extracts were treated with a few ml of Sodium Hydroxide solution (4%) followed by copper sulphate solution (1%). It turns into violet colour.

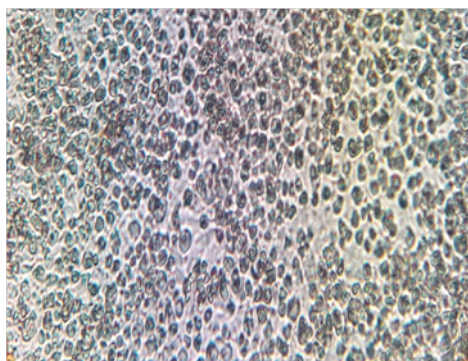
To the extract, 1 or 2 ml of Anthrone was added, the solution changes into a green coloured solution.

Saponins

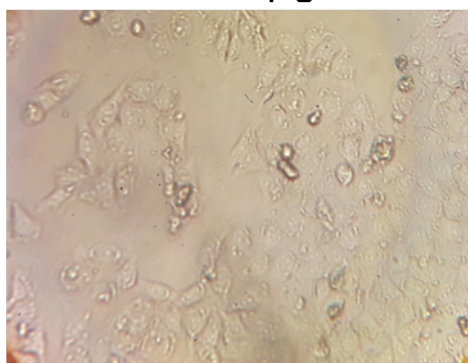
Few mg of ethanol and aqueous extracts were added to Na₂HCO₃ and shaken for about 5 mins which result in the formation of foam.



Control

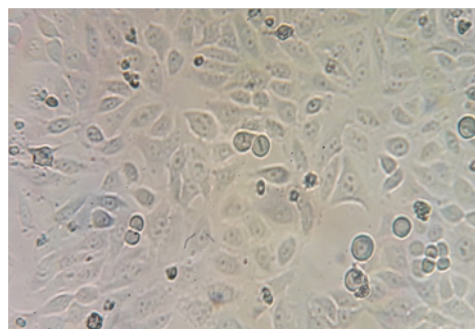


Conc. 100 µg/mL

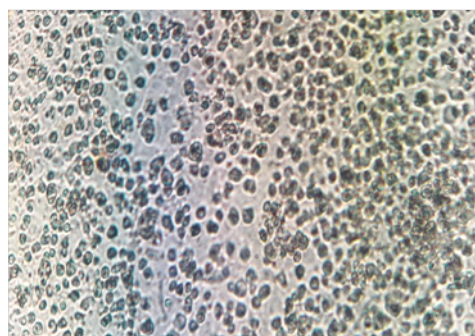


Conc. 200 µg/mL

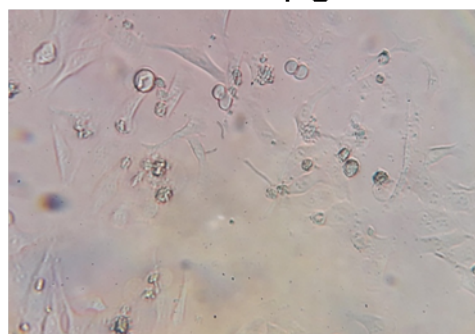
Figure 2: Anticancer activity of Aqueous Extract of *A.Scholaris* against the HeLa cell lines



Control



Conc. 100 µg/mL



Conc. 200 µg/mL

Figure 3: Anticancer activity of Ethanol Extract of *A.Scholaris* against the HeLa cell lines

Glycosides

Few ml of NaOH solution was added to 1mg of aqueous and ethanol extracts. The solution turns into Yellow coloured solution. Keller-killiani test: The plant extract was dissolved in 5ml of glacial acetic acid. To this, a few drops of ferric chloride solution and Conc. sulphuric acid was added, which turns into greenish-blue colour.

Steroids: Lieberman’s test

Chloroform was added to the extract and followed by the addition of concentrated H₂SO₄ slowly by the side of the test tubes.

Cytotoxicity Effect of *Alstonia Scholaris*

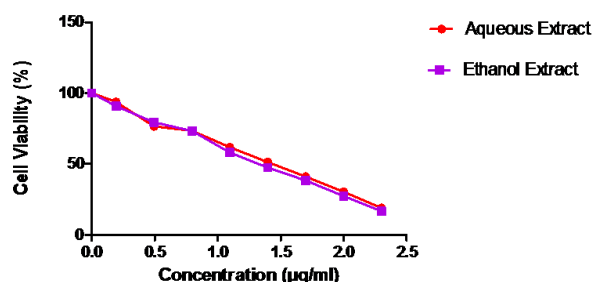


Figure 4: Cytotoxicity effect of *Alstonia scholaris* against HeLa cell

The lower part of the test tube solution turns into brownish yellow, and the upper part turns into reddish-orange colour.

Gums and mucilage

The plant extract was added to 5ml of distilled water, and few drops of ruthenium red solution were added. The solution turns into a pink colour.

INVITRO CYTOTOXICITY ASSAY

Preparation and seeding of cells

The HeLa cells were suspended in Dulbecco's Modified Eagle's Medium, which was trypsinized by discarding the culture medium. To the cells containing 25mL of DMEM, 10% FCS was added, and the cells were homogenized.

Cytotoxicity assay

The cytotoxicity assay was carried out using MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide) Reagent. MTT reagent is used to assess the cell viability or cytotoxicity as it was cleaved by mitochondrial enzyme succinate dehydrogenase to purple formazan product. 1mL of the homogenized cell suspension was added to 96 well plates. To these different concentrations of aqueous and ethanol extracts (0 to 200 μ g/mL) and were incubated at 37^oC in CO₂ incubator. After 48hrs, MTT was added to each plate and left for about 3hrs in room temperature. Later, the solution was removed using pipette and 100 μ l of DMSO was added to dissolve the formazan crystals. The absorbance was measured in Read Well Touch Microplate Reader at 570nm (Abondanza *et al.*, 2008; Mosmann, 1983).

RESULTS AND DISCUSSION

PHYTOCHEMICAL STUDIES

Qualitative phytochemical screening Table 1 of bark extract of *Alstonia scholaris* revealed the presence of major phytochemicals constituents such as Alkaloids, Glycosides, and Flavonoids etc.

In-Vitro cytotoxicity effect of *Alstonia Scholaris* against HeLa cell line

In HeLa cell line cytotoxicity effect was observed in tested sample concentrations in 48 hours treatment, it also revealed that increased concentration of drug shown increased cytotoxicity over the HeLa cell lines when compared to control (Figure 2 , Figure 3 and Figure 4). The IC₅₀ concentration against HeLa cell lines showed as 13.38 μ g/ml and 14.21 μ g/ml of aqueous and ethanolic bark extracts of *Alstonia Scholaris*, respectively Table 2.

CONCLUSION

In Women, Cervical cancer was found to be one of the reasons for death among them. Now-a-days, cancer treatment involves chemotherapy, radiotherapy and surgery. But these treatments would result in undesirable side effects, and the new emphasis is towards the plant-based products to treat cancer with reduced side effects. The qualitative phytochemical analysis showed the presence of Flavonoids, Alkaloids, and Glycosides etc. *In-Vitro* cytotoxicity assay (MTT assay) was performed, and the IC₅₀ value of those extracts was calculated. The IC₅₀ value of aqueous extract was found to be 13.38 μ g/ml, and ethanol extract was found to be 14.21 μ g/ml. Finally, it was proved that the bark part of *Alstonia Scholaris* showed a significant cytotoxic effect. Further studies will be performed on the isolation of phytoconstituents and performing molecular mechanism pathway to prove the plant as effective anti-cancer agents.

Conflict of Interest

The author has no conflict of interest to declare.

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