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Ethnobotanical and Pharmacological Study of *Alstonia* (Apocynaceae) - A Review

Kumar Pratyush , Chandra Shekhar Misra, Joel James , Lipin Dev M. S., Arun Kumar Thaliyil Veettil, Thankamani V*.

School of Bio Sciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India

Abstract:
The use of folkloric medicine has been the tradition of Indian therapeutics since time immemorial. Many plant and their parts have been used as a remedy for various diseases. *Alstonia* is one of the most important genus of Apocynaceae family to which many pharmacological activities can be attributed. The number of alkaloids obtained from plants like ditamine, echitamine have been used in various diseases like diarrhea, beri-beri, malaria and is till under detailed investigative study to bring about its potential medicinal properties. There are many reports about the various traditional uses to which this plant has been used for. Therefore this paper therefore aims to bring out the ethnobotanical uses of genus *Alstonia* with special significance to two most studies species viz. *Alstonia scholaris* and *Alstonia boonei* so as to provide better scope of carrying out more in vivo experiments based on evidences presented in this review.
Keywords: *Alstonia scholaris*, *Alstonia boonei*, Folkloric, Pharmacological, Review.

INTRODUCTION

Alstonia is a genus of the family Apocynaceae to which many other medicinally important plants belong like *Rauvolfia canescens* , *Alstonia boonei*, *Rauwolfia serpentine* and *Vinca rosea* which have been producing well known remedy for various disorders like schizophrenia and cancer. The traditional method of medications has been long known in the developing countries like India and China [1]. The important plants of genus *Alstonia* includes *Alstonia scholaris, Alstonia boonei, Alstonia congensis* and *Alstonia macrophylla* which have proved to be useful in various diseases. Almost all plant parts viz. leaves, stem bark, root and inflorescences have been used and are further under investigative study.

Pharmacological activities of various plants have continued to interest various pharmacists across the globe. Herbal plants and their extracts have shown the presence of various bioactive components which have demonstrated the wide range of activities from treating cough cold to cancer and other deadliest diseases.

Alstonia sp are tropical plants growing in various parts of Africa and south Asia. *Alstonia* is named after Professor Dr C. Alston (1685-1760), at department of botany in University of Edinburgh. More than 40 known species are there in which most studied species of genus *Alstonia* includes *Alstonia scholaris* and *Alstonia boonei.*

Alstonia scholaris also known as devil tree is an evergreen tree growing upto height of 100m. It is used against chronic diarrhoea, dysentery, bowel movements, beri-beri, congestion of liver, Dropsy and ulcers[2]..*Alstonia boonei* is commonly known as Ahun in Yoruba, Egbu-ora in Igbo, Ukhu in Edo and Ukpukunu in Urhobo [3]. It is used as therapeutics for dysentery, typhoid, gonorrhoea and asthma and is also applied to ulcers, toothache, snakebites, rheumatic pain and sores and as a galactagogue. Therefore the present review is focused on detailed study of ethano-pharmacological importance with respect to the bioactive compounds of the genus *Alstonia* with special emphasis to *Alstonia scholaris* and *Alstonia boonei.*

CHEMICAL COMPOSITION

The various species of *Alstonia* are highly rich in alkaloids, steroids and triterpenoids, and phenolic compounds which contribute to the toxicity of *Alstonia scholaris* [4].Various alkaloids that have been reported in stem bark of *A. scholaris* includes alstonidine, *O*-methylmacralstonine, macralstonine *O*-acetylmacralstonine, alstonine, ditamine, echicaoutchin, corialstonidine, corialstonine chlorogenine, villalstonine, pleiocarpamine, villalstonine, macrocarpamine, and triterpenoids which have been reported are alpha-amyrin linoleate, lupeol palmitate and lupeol linoleate[5, 6]. There have been several other alkaloids that had been isolated and reported which are 12-methoxyechitamidine, 5-epi-nareline ethyl ether, nareline methyl ether, scholaricine, picrinine, and scholarine-N(4)oxide [7], 19-hyroxytubotaiwine[8], 6,7-seco- 19,20-epoxyyanggustibobine B , Nb-methyl-scholarine, Na- metylburnamine, 19-epischolarine and vallesamine Nb-oxide[9], 19,20-[E]-vallesamine, angustilobine, 20(S)-tubotaiwine, B-N4-oxide, and 6,7-seco-angustilobine[10]. Leaves of *Alstonia scholaris* have been the source of new picrinine-types of mono-terpenoid indole alkaloids which are 5-methoxystrictamine, picralinal and 5-methoxyaspidophylline [11].

Alkaloids such as ditamine , echitamine and echitenine obtained from bark of *A. scholaris* are yellow colored amorphous mass. Acicular crystals

form of Echicerin and crystallized scales of echitin have been reported from bark extract. Similar alkaloids like echitein(a crystallisable acid) in rhombic prisms and an amorphous substance called echiretin are all like an alkaloid, a fatty acid and fatty resinuous substances [12]. Ditain which is an uncrystallisable bitter principle was isolated long ago and attributed to have antipyretic properties. Ditamine and echitamine can be extracted with ether and choloroform by making the solution alkaline with sodium bicarbonate and NaOH respectively. Echitamine ($C_{22}H_{25}O_4N_2$) is major alkaloidal constituent of several species of *Alstonia* like *A. angustiloba* , *A. gilletii*, *A. congensis* and *A. spathulata* including *A. scholaris,* however the same alkaloid was absent in other species of *Alstonia* like *A. villosa, A. constricta*, or *A.macrophylla*. Lupeol acetate, stigmasterol and α- sitosterol have been isolated from root bark [12].

Alkaloids such as chlorogenic acid and several other hallucinogenic indole alkaloids which have been reported in the seeds of *A.scolaris* are chlorogenine, alstovenine, reserpine, echitamine, ditamine, and venenatine. 7-megastigmene-3, 6, 9-triol and megastigmane-3β, 4α, 9-triol are the two important structures which have been identified and were extracted from the leaves of *Alstonia scholaris* and are known to be C_{13}-norisoprenoids [13]. Alstonic acids such as 2, 3-secofernane triterpenoids were also found to be isolated from leaves of *Alstonia scholaris* [14].

PHARMACOLOGICAL USES

FOLKLORIC USES

Alstonia scholaris is used as bitter tonic, aphrodisiac, febrifuge, stimulant, expectorant, alterative, carminative, anti- periodic, astringent and stomachic [12]. For ages, the plant parts have been used in the treatment of chronic diarrhea, fevers, dysentery. Ayurveda has acknowledged the use of bark asalterative, tonic and gastro-intestinal sedative and have been used as suitable alternative to quinine. Ditamine has anti-periodic properties, though its anti-pyretic property is not lasting.

The bark extract is also a useful remedy for treating asthma, lung cancer, hypertension, and pneumonia while the leaf extracts is used against fever [15]. It is effective against boils and ulcers and can be cured by applying milky latex or young leave as poultice over affected area.

For chronic diarrhea, and fever 1% extract of bark is used as tea. Malaria is treated by using 5% bark decoction as tea. Oil mixed with milky juice, can be used for relieving ear aches. Tincture of the bark can be used as strong galactagogue in some cases. Extract of the bark is used as anticholeric, emmenagogue, and vulnerary.

The bark of *A.Scholaris* is also given to lactating mothers to increase lactation and overcome some of the major post delivery weakness and digestion. Fresh ginger roots or zedoary mixed with leaves juice is given to women after confinement [12]. Roots however have been reported to have very rare medicinal use in enlarged liver and pain but no therapeutic intervention is available so far.

Table 1: Folkloric uses of *Alstonia scholaris* Linn. R.Br.

Parts	*Uses*	*References*
Bark	Tonic, aphrodisiac, febrifuge, stimulant, expectorant, alterative, carminative, anti-periodic, astringent and stomach ache. Used to treat leprosy, dyspepsia, malarial fever, Leishmania infection.	12
Milky Juice or latex of Bark	Pimple, dental caries (pyorrhoea).	12
Tender Leaves	Leaves pulverised to make poultice in treatment of ulcers. Used in snake bite and scorpion bite.	16
Leaves	Used for treating ulcer, rheumatic pain, asthma and diabetes	2
Flower	Used in asthma and other respiratory problems	17
Roots	Used in enlarged liver with pain	17

STUDIES AND PRE-CLINICAL DATA

Broncho-Vasodilatory Activity

The ethanolic extract of the leaves of *Alstonia scholaris* has shown pronounced broncho-vasodilatory effect. Administration of the plant extract at various concentrations like 25mg/kg, 37mg/kg and 50 mg/kg to anaesthetised rats at 5 min before the administration of broncho-constrictor carbachol (10 μM/kg) led to a decrease in the normal blood pressure of the animals by 54±13% and 81±7% at doses of 25 and 37 mg/kg, respectively. In contrast the inspiratory pressure was increased by 50±13% and 83±12% at the concentration of 25 and 37 mg/kg respectively, while the expiratory pressure and heart rate remained unchanged. The high dose of 50 mg/kg of the leaf extract caused a rapid decrease in the

blood pressure and the rhythm of respiration was altered [15].

The broncho dilatory effect of plant extract can not be due to dilation of bronchial muscle because no activity was found for the same in in-vitro experiments conducted on guinea pigs. The vasodilatory activity of the leaf extract was mainly attributed to nitric oxide, and was found to be independent of adrenergic or muscarinic receptors or prostaglandins [15]. This bronchodilatory activity of extract was found to be due to presence of various chemical constituent in *Alstonia scholaris* which include akaloids such as alstonamine and rhazimanine [18] picrinine [19], schloaricine [20], or triterpenes (betulin, ursolic acid) or sitosterol [21].

Thus the bronchodilatory effect of ethanolic extract of leaves of *Alstonia scholaris* can be sourced as a popular use of this plant to treat various cardiovascular, gastrointestinal and several respiratory disorders.

Anticancer Activity

The HeLa cells were used to investigate the cytotoxicity of extracts of *A. scholaris* which was found to be dependent on the season in which it was collected be it the summer ,monsoon, or winter [10]. The bark collected in the summer was most toxic followed by that collected in the winter and the least toxic was in the monsoon season. Cytotoxicity was tested with variable doses of various fractions from extract of *A. scholaris* and was found to be highest for the residue fraction and lowest for the steroidal fraction. Cytotoxic effect, in the order of most toxic to least toxic, was the residue fraction followed by whole extract then chloroform extract, echitamine chloride, followed by ethyl acetate fraction then diethyl ether fraction, followed by petroleum ether fraction then n-butanol fraction, then aqueous fraction and the least toxic being steroidal fraction [10] .

One of the important alkaloid present in *A.scholaris* called alstonine was reported to have antitumor activity in YC8 lymphoma and Ehrlich ascites carcinoma cells.Bisindole and villalstonine as reported by [22] showed marked activity against human cancer cell lines, COR-L23 (large cell carcinoma) cell line and MOR-P (human lung adenocarcinoma). However alkaloids like pleiocarpamine, macralstonine and *O*-methylmacralstonine were much less active as compared to villalstonine. Several alkaloids like *O*-acetylmacralstonine, macrocarpamine and villalstonine have been reported to be cytotoxic to human cancer cell lines, MCF7 (breast adenocarcinoma), StMI1 1a (melanoma), Caki-2 (renal cell carcinoma), COR-L23, MOR-P and LS174T (colon adenocarcinoma) [10, 23].

The hydroalcoholic extract of *A. scholaris* was studied to determine its chemopreventive ability on benzo(a)pyrene-induced fore stomach carcinoma in female mice at different concentrations of 1, 2, and 4 mg/ml when added to drinking water for 2 weeks before, during and 2 weeks after the carcinogen treatment. The administered doses thus reduced tumour multiplicity by 21.43, 28.57 and 50%, respectively. The greatest protection was observed in the case of the highest dose of 4mg/ml which reduced tumour occurrence by 6.67% [6]. Thus 4mg/ml dose was found to significantly reduce the tumour multiplicity incidence by 91.93% when compared to BaP treated (100%) animals. The hydroalcoholic extract was able to inhibit benzo(a)pyrene-induced mutagenic changes as evidenced by the number of splenocytes bearing one micronuclei and the one bear multiple micronuclei were reduced by the extract. Pretreatment of mice with 4mg/ml of the extract showed no sign of the tumor multiplicity and tumor incidence [6].

The extract of *A. scholaris* has been recently reported to exhibit immunostimulatory activity and was able to enhance phagocytic effect in normal and immunosuppressed mice [24]. Thus it may be concluded that the immunomodulation may also have played an important part in the observed chemopreventive activity of*A. Scholaris* [6]. The alcoholic extract of the stem bark has been reported to possess anticancer activity in HS1 human sarcoma in embryonated egg [5]. An investigation with 85% ethanolic bark extract of *A. scholaris* showed antitumor and radiation sensitising activity against a mouse transplantable tumor and is also found to be toxic to human tumour cell lines in in-vitro condition [5]. Efficiency of *Alstonia scholaris* in enhancing the anticancer activity of Berberine in the Ehrlich Ascites Carcinoma-Bearing Mice was found to be more in the early stages than in later tumor developmental stages [25].

Radioprotective or Anti-Mutagenic Effect

The hydroalcoholic extract of the bark of *Alstonia scholaris* has been studied for its radiopreventive efficiency in mice against radiation induced biochemical and haematological alterations [26]. Mice administered with the *Alstonia scholaris* extract for 5 consecutive days prior to the gamma irradiation. Exposure to radiation however resulted in the lowering of the erythrocytes and haemoglobin until third day, thereafter showing signs of recovery, but these values failed to reach the normal for the rest of the animal's life. Animals pretreated with extract showed higher erythrocyte count, hematocrit and hemoglobin

values than their corresponding irradiated controls. Lower lipid peroxidation level, and higher glutathione in serum and liver was found compared to their irradiated controls. Thus *Alstonia scholaris* was found to be protective against the haematological and biochemical changes in mice which were caused by radiation [26].

Antimalarial Activity

The various alkaloids from *Alstonia scholaris* have been found to be effective against malaria like bisindole alkaloids including villalstonine and macrocarpamine which are active against multi-drug resistant K1strain of *Plasmodium falciparum* [27]. Other alkaloids like Corialstonine and corialstonidine, obtained *Alstonia scholaris*, are also found to be active against *P. falciparum* [10]. No antiamalarial activity was evidenced in mice infected with *Plasmodium berghei* when administered with the petroleum ether extract and methanol extract of the bark of *Alstonia scholaris.* However, the animals showed the sign of improvement and delayed mortality when methnol extract of A. *scholaris* was given in a dose-dependent manner [28].

The antiplasmodial activity was tested for two plants *Morinda lucida* and *Alstonia boonei.* The antimalarial activity was found to reside predominantly in N- Hexane and Chloroform fractions, however at same concentration, former was found to exert higher activity than the latter. The crude ethanol extracts of the stem bark of *Alstonia boonei* inhibited the schizont of *Plasmodium falciparum.* The minimum inhibitory concentration required for inhibiting was found to be 0.2 mg/ml. However , the MIC for other extract was found to be as follows; N Hexane 0.07mg/ml,Chloroform 0.07mg/ml, ethyl acetate 1.71.7mg/ml and butanol 0.6mg/ml. they were tested against the standard antimalarial drug Chloroquine for which MIC was found to be 0.6µg/ml [29].

The anti plasmodial activity of the methanol extracts of various parts of *A.Scholaris* have been evaluated by Keawpradub et al against multi drug-resistant K1 strain of *Plasmodium falciparum* cultured in 73 human erythrocytes. Pronounced antiplasmodial activity was exhibited. The indole alkaloids were isolated from the active extract and were subsequently tested against the K1 strain of *P. falciparum* and were found to have pronounced antiplasmodial activity mainly among the bisindole alkaloids, mostly villalstonine and macrocarpamine with IC_{50} values of 0.27 and 0.36 µM, respectively [27]. However Gandhi and Vinayak have reported that bark of *Alstonia scholaris* extracted with petroleum ether and methanol were found to have no anti malarial activity in mice infected with *Plasmodium berghei*. But they have noticed an improvement of conditions and delayed mortality amongst animals receiving methanol extract of *A. scholaris* in a dose dependent manner [28].

Studies also stated that the feeble effect of *A. scholaris* was found in malaria induced in monkeys and naturally occurring in human patients. Therefore it cannot be recommended as an absolute substitute for quinine and other cinchona alkaloids for malarial treatment [2]. Trials were made against bird malaria using alkaloid akuammine, distamine, echitamine, and harmine but produced no effect except echitamine which showed weak activity even in doses of 5mg , thus reported the ineffective alstonine sulphate in bird malaria [12].

Similarly the alkaloid 'echitamine' did not show any anti-protoplasmic property like quinine or emetine as suggested by the study on Amoebae being suspended in 1% of the echitamine hydrochloride solution showing no sign of mortality even after 2 hours of exposure. Thus the use of this plant extract instead of quinine as a potent anti-malarial or for amoebic dysentery would thus be doubtful and will have lesser pharmacological importance. Slight action can be expected from this alkaloid at the dose of 5mg [12].

Antifertility Activity

Bark extract of *A. scholaris* when administered orally into male rats for 60 days at doses 200 mg/day showed significant decrease in fertility. There was marked reductions in the weights of epidydemes, testes, seminal vesicle and ventral prostate [30]. Spermatids production was found to be reduced by 79.6 % while the population of spermatocytes which were in their preleptotene and pachytene stage was decreased by 61.9 % and 60.1%, respectively. Several other indications of male fertility were observed like sertoli cell population, spermatogonia, and areas of the Leydig cell nuclear and seminiferous tubule were significantly reduced [30].

Anti-Diabetic

Compared to *Cratoxylum mangayi* and *Dillenia indica* the leaves of *Alstonia scholaris* have shown more inhibitory effect for two sugar which are sucrase and maltase [31]. Powdered *Alstonia scholaris* leaves were used to assess the hypoglycaemic effect. 30 ml of plain water and 1.0 g of the powdered leaves along with 30 ml water was given to group A and group B respectively each comprising of 6 normal person while 1, 2 and 3 g of the powdered *A. scholaris* leaves were given to groups C, D and E

comprising of 6 Non Insulin Dependent Diabetes Mellitus patients each and a 2.0 mg tablet of standard Amaryl (sulphonylurea drug) was used as a positive control which was administered once a day to 6 NIDDM patients of Group F. Blood glucose levels of all test subjects were determined 2 hours after food, on the post-treatment days 0, 1, 8 and 15. The result showed that the normal person with oral adminsteration of *Alstonia* powder had decreased blood glucose level. However, in NIDDM patients, treatment with 3 g of the powder to group E showed a highly significant reduction in blood glucose. While control results with one 2 mg Amaryl tablet each to Group F also showed a highly significant reduction in blood glucose levels. It can therefore be agreed, that in the patients with NIDDM, powdered *Alstonia scholaris* leaves exhibits hypoglycaemic effect. Thus the plant drug from *Alstonia* can be insulin triggering and can direct insulin-like actions [32].

A study was also conducted on 24 traditional medicinal plants which showed the presence of potent α-Glucosidase inhibitory activity of methanol extracts of leaves of Devil tree(*A. scholaris*). The structures of the active compounds against α-Glucosidase were found to be quercetin 3-*O*-β-D-xylopyranosyl (1‴ → 2″)-β-Dgalactopyranoside and (−)-lyoniresinol 3-*O*-β-D-glucopyranoside [31]. Lyoniresinol 3-*O*-β-D-glucopyranoside was found to have inhibitory effect on both maltase and sucrase whereas quercetin 3-*O*-β-D-xylopyranosyl (1‴ → 2″)-β-D galactopyranoside was inhibiting only maltase. Thus the present preliminary study showed formed the strong basis regarding the suitability of using *Alstonis scholaris* as an important medicinal source for treatment and prevention of diabetes [31].

Immunostimulatory

To study the immuno stimulatory effect in mouse, bark extracts of *Alstonia* were given orally, once a day for 7 consecutive days. The results of the study revealed that at the same doses the aqueous extract had higher phagocytic index than the ethanolic extracts in normal mice. The aqueous extract also increased the phagocytic activity of immunosuppressed mice. The aqueous extract at 100 mg/kg body weight significantly increased lytic activity of peritoneal exudate cells against *Escherichia coli*. At lower doses the aqueous extract had no effect on the level of primary antibody. However the cellular immune response was induced by the aqueous extract at 50 mg/kg b.w. while at 100 mg/kg b.w. had negative impact on the delayed type of hypersensitivity reaction [24].

Antioxidant / Free Radical Scavenging

Alstonia scholaris extracted with ethanol showed antioxidant properties with significant free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging and significant ferric thiocyanate reducing activities and were comparable to the standard antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), l- ascorbic acid and α-tocopherol [33]. The study was conducted on 17 Indian medicinal plants to evaluate their regulatory effect on nitric oxide levels and was found that the bark of *A. scholaris* had the most potent scavenging activity with regards to nitric oxide [34].

Antioxidant activity of *Alstonia boonei* was tested using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, total phenol content and reducing power which were found to be 41.58 ± 1.43 %, 2.09 ± 0.04 mg/g gallic acid equivalent and 0.32 ± 0.01 respectively. The phytochemical analysis was expected to have phenolics as a major compound which would provide high antioxidant activity but study indicated that opposite was the case. The plant showed low antioxidant indices [35].

Analgesic And Anti-Inflammatory Activities

Experimental models of pain and inflammation were used to study the effect of ethanolic extract of leaves of *Alstonia scholaris* [36]. Significant decrease was observed when the leaf extract (200 and 400 mg/kg)was administered to acetic acid induced pain in mice. The percentage of pain inhibition in hot plate method was found to be 73.90 % and 79.56 % with 200, 400 mg/kg of extract. Carrageenan induced paw oedema was greatly reduced with 200 and 400 mg/kg of the extract.

Anti-Ulcer Activity

Pyloric ligation method was used to evaluate the ethanolic extract of leaves of *Alstonia scholaris* for anti-ulcer activity [36]. The result showed that the animals which were treated with the extract showed no signs of ulcer, as compared to the rat which developed the ulcer score upon the administration of diclofenac sodium.

Wound Healing Activity

Excision, incision and dead space wound models were used against which the wound healing activity of the ethanol and aqueous extracts of *Alstonia scholaris* was tested [37]. The mechanism of wound healing was studied with respect to the rate of wound contraction, skin breaking strength, granulation strength, period of epithelialisation, hydroxyproline, dry granulation tissue weight, collagen and histopathology of granulation tissue. Extent of lipid peroxidation level was also

evaluated using malondialdehyde level. The extracts of *A.scholaris* promoted wound healing significantly in all the wound models under study. However increased rate of wound contraction, granulation strength, skin breaking strength, hydroxyproline, dry granulation tissue weight and collagen, and decrease in the period for epithelialisation and increased collagenation in histopathological section were observed in the groups which were treated with extracts. The levels of lipid peroxidation was also decreased.

Hepatoprotective Activity

The *Alstonia scholaris* has a significant role in hepato preventive measures on liver injuries caused by acetaminophen, carbon tetrachloride, ß-D galactosamine, and ethanol. The hepato protective activity was investigated by Lin *et al* by serum-biochemical and histopathological examinations. All results including both serological and histopathological changes of *A. scholaris* were similar to those of *Bupleurum chinense*, which had been previously reported as a useful remedy for hepatitis. Histopathological examination studies revealed that ß-D galactosamine inhibit cell necrosis and inflammatory cell infiltration [38].

Antimicrobial Activity

The antimicrobial properties of various parts of plants viz. leaves ,stem bark and root bark of *Alstonia scholaris* have been studied using different solvent system each showing wide range of antimicrobial activity against both gram positive and gram negative bacteria. It has been reported that the chemical constituents of *Alstonia scholaris* (alkanes, sterols and alkanols) were responsible for its antimicrobial property[39]. Butanol fraction exhibited the broad spectrum of antimicrobial activity as compared to other solvent system like petrol, dichloromethane, ethyl acetate, butanol fractions of leaves,stem bark and root bark of *Alstonia scholaris* [40].Similarly methanol leaves extract showed broad spectrum antimicrobial activity against the test organisms as compared to chloroform and acetone while no inhibitory effect shown by petroleum ether extract [41]. Comparative study on antimicrobial activity of Leaves, stem bark and roots was done and was found that maximum zone of inhibition was found in methanolic root extract of *Alstonia scholaris* and most inhibited was *E.coli*. Similarly the flowers of the plant were tested for their antimicrobial property and was found to be potent antimicrobial agent [42, 43].

Antimicrobial activities of aqueous and ethanolic extracts of stem bark of *Alstonia boonei* were also tested to screen for their antimicrobial properties using agar diffusion method and was found to be dose dependent. The phytochemical screening of the plant extract showed not only the present of bioactive componts like alkaloids, flavanoids, steroids but it also contained macro elements like calcium, magnesium, sodium, potassium and copper to varying degree. It was also found that active components were present more in the ethanolic extract than in the aqueous. The LD50 was estimated to be 6.17 g/kg, and doses higher than this showed lethal effect on 50% of the population. The maximum zone of inhibition was given in case of *Candida* sp by the aqueous extract at a dose of 1000 mg/ml while ethanolic extract gave the highest inhibition zone at 1000mg/ml on *Bacillus subtilis* and *Pseudomonas aeruginosa.* Though the standards used were ciprofloxacin and chloramphenicol which gave higher inhibition at much lower concentration [44]. Minimum inhibitory concentration for ethanol extract was found to be 250 mg/ml for all organisms used except *Pseudomonas aeruginosa* which was resistant to this concentration, but was inhibited at higher concentration of 500mg /ml. The MIC value the aqueous extract was 500 mg/ml for all the organisms except for *Pseudomonas aeruginosa* for which extract concentration more than 500 mg/ml is required to inhibit [44].

Herbicidal Property

The aqueous extract of *A.scholaris* has been found to exhibit phytotoxic activity on parthenium which showed delayed seed germination as well as reduced the final germination percentage of parthenium. The extract was found to be highly toxic and even the lowest concentration of 2% of the extract caused significant reduction in the final germination by 30% when compared with control. Further increase in extract concentration resulted in a corresponding decrease in germination. The 10% extract reduced the germination by 80% as compared with control.

Thus the present study concluded that aqueous leaf extract of *A. scholaris* contain potent herbicidal constituents for the management of parthenium weed which supported the findings that the seedling growth of parthenium can be checked by aqueous extracts of allelopathic plant species [45].

Anti –aging function of Retinoids

The study was done on ethanol bark extract of *Alstonia scholaris* which showed that it could significantly inhibit all trans retinoic acid – induced inflammation in human Ha Cat keratinocyte cells. In vitro dose dependent suppression was observed in the two representative retinoid-induced pro-inflammatory cytokines, monocyte chemo attractant protein-1 and interleukin-8, by *A. scholaris* extract by 82.1% and 26.3% at 100 ppm. It was also found that in a

cumulative irritation patch test, *A. scholaris* extract decreased retinol-induced skin irritation, while strengthening the ability of retinoids to inhibit matrix metalloproteinase-1 expression, which was strongly associated with aging effects. Thus it was suggested that *A. scholaris* had the potential to increase the anti-aging function of retinoids and also reducing their ability to cause skin irritation [46].

INSTRUMENTAL ANALYSIS

In order to identify the role of functional group which contribute to the various activities exhibited by the plant extract, the spectral analysis of the plant extract was done. In order to draw the comparison and shift in the spectrum, the FT-IR of dried powdered methanolic root extract was done followed by the *E.coli* cell pellet before and after being treated with methanolic root extract. Among the various functional groups involved, Alkanes, Alkenes, Nitro compounds, Alkynes, Phenols, Carboxylic acids , Amines played a major role in interaction of extract with *E.coli* cells [47].

Cytotoxicity assays

The cytotoxic effect of methanolic root extract of *Alstonia scholaris* on mouse fibroblast cell lines was evaluated using MTT assay. The sample was found to cause lethal cytotoxic effect at a dose of 1mg/ml when the exposure period was 24 hrs. But at longer exposure periods i.e., 48, 72 and 96 hrs the extracts showed no cytotoxic effect.. However, other lower concentrations were found to be non-cytotoxic in L929 mouse fibroblast cells under experimental conditions [47].

Anti-Diarrheal

The pharmacological evaluation of the crude extract of *Alstonia scholaris* was done which showed that the presence of alkaloids, provided 31-84% protection against castor oil-induced diarrhoea in mice at the concentration of 100-1000 mg/kg doses. The detailed study revealed spasmolytic activity which was mainly due to calcium channel blockade (CCB) which was later confirmed by pre-treatment of the tissue with the *A. scholaris* (0.3-1 mg/mL) showing effect similar to the verapamil, a standard calcium channel blocker. These results were the clear indication of the fact the antidiarrhoeal and spasmolytic effects were exhibited by the crude extract of *Alstonia scholaris*, which may possibly attributed to the presence of CCB-like constituent(s) and thus serves as a base for its medicinal use in diarrhoea and colic [48].

Anti-Tussive, Anti-Asthmatic and Expectorant

Study was carried out to find out Anti-tussive and anti-asthmatic activities of the ethanolic fraction of *A.scholaris* leaf which could provide a strong evidence for its clinical use. The alkaloids present in the alcoholic fraction significantly reduced the frequency of coughing in mice in all the three models including ammonia or sulfur dioxide induced mice coughing [49]. The alkaloids fraction decreased the occurrence of convulsion, and the expectorant activity which was estimated based on the volume of phenol red in the trachea of mice showed enhanced output of phenol red in trachea. Moreover, *in-vivo* anti- tussive and anti-asthmatic activities was exhibited most by the picrinine alkaloid which can be put in quality control of the products from *Alstonia scholaris* leaf. Thus the alkaloids fraction from *Alstonia scholaris* leaf was found to exhibit anti-tussive, anti-asthmatic and expectorant properties and hence serve as a valuable lead material for respiratory disorders drug development [49].

SIDE EFFECTS

Toxicity Studies

The leaves of *Alstonia scholaris* when extracted with ethanol (30, 300, 1000 and 2000 mg/kg body weight) and administered in to the mice through intraperitoneal route did not cause any alterations in the behavior and autonomic responses when compared to controls. Following the administration of extract, no mortality was observed upto 2g/kg of the extract during the 48 hour observation period [15]. Season dependent acute toxic effects were obtained in case of the hydroalcoholic extract of the stem bark of *A. scholaris*, in which the most toxic was the bark collected in the summer (LD_{50} of 900 mg/kg), followed by bark collected in the winter (LD_{50} of 1075 mg/kg) while the least toxic extract was monsoon season extract (LD_{50} of 1200 mg/kg) [5]. Susceptibility to toxicity varied according to the mice strain, the most susceptible being Swiss albino mice (all animals died at 1100 mg/kg) while crossbred mice were resistant. Intraperitoneal administration caused more lethality when compared to oral route. Extract administered orally was toxic only beyond 2000 mg/kg body weight. However extract given through intraperitoneal route led to the maximum number of dead animals even at a dose of 1100 mg/kg. Rats were given the daily doses of 120 and 240 mg/kg for 30 days (corresponding to 1/10th and 1/5th of the LD_{50} dose) to estimate the subacute toxicity. However with the rats receiving lower doses showed no changes in physiological activities, general behaviour, or final body weights and no mortality was reported. In contrast the higher dose being toxic caused lethargy in the rats, 30% mortality, a significant reduction in the final body weight and formation of various deformed organs. Higher

dose of *A. scholaris* extract had more lethal impact on males than females. Thus the administration of higher doses of *A. scholaris* should be dealt with extreme care as it can lead to severe damage to all major organs of the body [5].

Hydroalcoholic extract of a 1:1 mixture of *A. congensis* bark and *X. aethiopica* fruits were used to evaluate acute and subacute toxicity in Swiss albino mice. Extract were fed between the doses of 1.0 to 20.0 g/kg body weight and observed continuously for the first 4 h and for every hour for the next 24 h, then 6 hourly for 48 h. Wistar rats were also fed with different doses of the extract for 30 days to see the effects on biochemical parameters using subacute toxicity model. The LD_{50} of the extract was found to be above 20.0 g/Kg body weight. The result showed that plasma glucose and low density lipoprotein (LDL) levels reduced showing good hypoglycaemic effect whereas high-density lipoprotein (HDL)-cholesterol level was found to be increase in the treated animals. Lower doses of extract did not affect Aspartate aminotransferases (AST) and alanine aminotransferases (ALT) levels but creatinine levels was increased in all the treated animals. The acute study showed no evidence of drug-induced symptoms or death at all the doses of the extract administered but subacute results revealed a tendency to cause kidney problems on a long-term use [50].

Teratogenic Effects

Teratogenic effects were produced in mice when treated with the hydroalcoholic extract of *Alstonia scholaris* at doses greater than 240 mg/kg when exposed on day 11 of gestation. However the lower doses of the extract at 60, 120, 180, and 240 mg/kg did not cause congenital malformations, mortality, or alter the normal growth patterns. Similarly higher doses of more than 360 or 480 mg/kg caused a dose dependent increase in mortality, growth retardation and congenital malformations, characterized mainly by bent tails and syndactly. Higher doses also had a significant effect on eye opening, pinna detachment vaginal opening and also delayed fur development incisor eruption and testes descent [51].

CONCLUSION

The present information on *Alstonia sp* match with the scientific findings of the various folklore use literature available. The traditional method of medicine has gained momentum in the recent past owing to the rapid development of the isolation and characterization techniques and the advancement in the in vivo testing have led to the interest in the plant to be used a potential future drugs. The important compounds isolated from the *Alstonia sp* have a wide range of pharmacological activities which need to be studied in detail so as to establish their therapeutic potential and therefore the scientific authentication of these medicinal properties is indispensable which will substantiate the use of these plants for the future.

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REFERENCES

[1]. Dhivya, S., Rajeev Kumar, S., Ramachandran, V.S., Sathishkumar, R. Conventional and novel DNA barcodes for Apocyanaceae. Barcode of Life, Mexico, 2010.

[2]. Nadkarni, A. K. and Nadkarni, K. M. *Indian Materia Medica, Vol. I*, Popular Prakashan, Bombay 1976, pp 80-83.

[3]. Majekodunmia, S.O., Adegokeb, O.A. and Odekua, O.A. Formulation of the extract of the stem bark of *Alstonia boonei* as tablet dosage form. *Tropical Journal of Pharmaceutical Research*. 2008, *7 (2),* 987-994.

[4]. Singh, S.K. and Singh, A. Molluscicidal and anti-cholinesterase activity of *Alstonia scholaris* plant against freshwater snail *Lymnaea acuminata*. *Pakistan Journal of Biological Sciences*. 2003, *6*(16), 1442-1446.

[5]. Baliga, M.S., Jagetia, G.C., Ulloor, J.N., Baliga, M. P., Venkatesh, P., Reddy, R., Rao, K.V.N. M., Baliga, B.S., Devi, S., Raju, S.K., Veeresh, V. , Reddy, T.K, Bairy, L.K. The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna *(Alstonia scholaris)*in mice and rats. *Toxicology Letters*. 2004, *151*, 317-326.

[6]. Jagetia, G.C., Baliga, M.S., Venkatesh P. Effect of Sapthaparna (*Alstonia scholaris Linn*) in modulating the benzo(a)pyrene-induced forestomach carcinogenesis in mice. *Toxicological Letters*. 2003, *144*, 183-193.

[7]. Banerji, A. and Siddhanta, A.K. Scholarine: An indole alkaloid of *Alstonia scholaris*. *Phytochemistry*. 1981, *20*, 540-542.

[8]. Yamauchi, T., Abe, F., Chen, R.F., Nonaka, G.I., Santisuki, T., Padolina, W.G. Alkaloids from the leaves of *Alstonia scholaris* in Taiwan, Thailand, Indonesia and Philippines, *Phytochemistry*. 1990, *29*, 3547-3552.

[9]. Yamauchi, T., Abe, F., Padolina, W.G., Dayrit, F.M. Alkaloids from leaves and bark of *Alstonia scholaris* in the Philippines. *Phytochemistry*. 1990, *29*, 3321-3325.

[10]. Jagetia, G.C. and Baliga, M.S. The effect of seasonal variation on the antineoplastic activity of *Alstonia scholaris R. Br*. in HeLa cells. *Journal of Ethnopharmacology*. 2005, *96*, 37-42.

[11]. Cai, X. H., Liu, Y. P., Feng, T., Luo,X.D. Picrinine-type alkaloids from the leaves of *Alstonia scholaris*. *Chinese Journal of Natural Medicines*. 2008, *1*, 6, 20-22.

[12]. Singh, M.P., Panda, H. *Medicinal Herbs with Their Formulations*. *Daya Publishing house*, Delhi 2005, pp 88-90.

[13]. Yan, X.U., Feng, T.,Cai, X. H., Luo,X.D. A new C_{13}-Norisoprenoid from leaves of *Alstonia scholaris*.

Chinese Journal of Natural Medicines. 2009, *1*, 7, 21-23.

[14]. Wang, F.,Ren, F.C.,Liu, J.K. Alstonic acids A and B, unusual 2,3-secofernane triterpenoids from *Alstonia scholaris*. *Phytochemistry*. 2009, *5*, 70, 650-654.

[15]. Channa, S., Dar, A., Ahmed, S., Rahman, A. Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. *Journal of Ethnopharmacology*. 2005, *97*, 469-476.

[16]. Kirtikar, K.R. and Basu, B.D. *Indian Medicinal Plants*, Vol. II, Dehradun 1980, pp 111-114.

[17]. *The Wealth of India, Raw Materials*, Vol. I, CSIR, New Delhi 2004, pp 50 – 51.

[18]. Rahman, A., Alvi, K.A. Indole alkaloids from *Alstonia scholaris*. *Phytochemistry*. 1987, *26*, 2139–2142.

[19]. Chatterjee, A., Mukherjee, B., Ray, A.B., Das, B. The alkaloids of the leaves of *Alstonia scholaris* R.Br., *Tetrahedron Letters*. 1965, *41*, 3633–3637.

[20]. Rahman, A., Asif, M., Ghazala, M., Fatima, J., Alvi, K.A. Scholaricine, an alkaloid from *Alstonia scholaris*. *Phytochemistry*. 1985, *24*, 2771–2773.

[21]. Banerji, A., and Banerji, J. Isolation of pseudo-akuammigine from the leaves of *Alstonia scholaris* R.Br. *Indian Journal of Chemistry Section 15 B*. 1977, 390–391.

[22]. Keawpradub, N., Houghton, P.J., Eno-Amooquaye, E., Burke, P.J. Activity of extracts and alkaloids of thai Alstonia species against human lung cancer cell lines. *Planta Medica*. 1997, *63*, 97–101.

[23]. Keawpradub, N., Eno-Amooquaye, E., Burke, P.J., Houghton, P.J. Cytotoxic activity of indole alkaloids from *Alstonia macrophylla*. *Planta Medica*. 1999, *65*, 311–315.

[24]. Iwo, M.I., Soemardji, A.A., Retnoningrum, D.S., Sukrasno,U.M. Immunostimulating effect of pule (*Alstonia scholaris L. R.Br*., Apocynaceae) bark extracts. *Clin. Hemorheol. Microcirc*. 2000, *23*, 177-183.

[25]. Jagetia, G.C., Baliga, M.S. Effect of *Alstonia scholaris* in enhancing the anticancer activity of Berberine in the Ehrlich Ascites carcinoma-Bearing Mice. *Journal of Medicinal Food*. 2004, *7*, 2, 235-244.

[26]. Gupta, U., Jahan, S., Chaudhary, R., Goyal, P.K. Amelioration of Radiation- induced Hematological and biochemical alterations by *Alstonia scholaris* (a Medicinal plant) extract. *Integrative Cancer Therapies*. 2008, *7*, 3, 155-161.

[27]. Keawpradub, N., KirbyKirby, G.C., Steele, J.C.P., Houghto, P.J. Antiplasmodial activity of extracts and alkaloids of three Alstonia species from Thailand. *Planta Medica*. 1999, *65*, 8, 690–694.

[28]. Gandhi M., and Vinayak, V.K. Preliminary evaluation of extracts of *Alstonia scholaris* bark for in vivo antimalarial activity in mice. Journal *of Ethnopharmacology*. 1990, *29*, 51-57.

[29]. Bello, I.S., Oduola, T., Adeosun, O.G., Omisore, N.O.A., Raheem, G.O. and Ademosun, A.A. Evaluation of Antimalarial Activity of Various Fractions of *Morinda lucida* Leaf Extract and *Alstonia boonei* Stem Bark. *Global Journal of Pharmacology*. 2009, 3 (3), 163-165.

[30]. Gupta, R.S., Sharma, R., Sharma, A., Bhatnager, A.K., Dobhal, M.P., Joshi, Y.C., Sharma, M.C. Effect of *Alstonia scholaris* bark extract on testicular function of Wistar rats. *Asian J. Androl*. 2002, *4*, 175-178.

[31]. Anurakkun N. J., Bhandari M. R., Kawabata J. α-Glucosidase inhibitors from Devil tree (*Alstonia scholaris*). *Food Chemistry*. 2007, *103*, 4, 1319-1323.

[32]. Akhtar, M.S., and Bano, H. Hypoglycemic effect of powdered *Alstonia scholaris* (Satona). *Professional Med J*. 2002, *9*, 3, 268-271.

[33]. Arulmozhi, S., Mazumder, P.M., Ashok, P., Narayanan, L. S. In Vitro Antioxidant and Free Radical Scavenging Activity of *Alstonia scholaris Linn. R.Br*. *Iranian Journal Of Pharmacology & Therapeutics*. 2007, *6*, 191-196.

[34]. Jagetia, G.C. and Baliga, M.S. The evaluation of Nitric Oxide Scavenging Activity of Certain Indian Medicinal Plants *In Vitro*: A Preliminary Study. *Journal of Medicinal Food*. 2004, *7*, 3, 343-348.

[35]. Akinmoladun, A.C., Ibukun, E. O., Afor, E., Akinrinlola, B.L., Onibon,T.R., Akinboboye, A.O., Obuotor, E.M. and Farombi, E. O. Chemical constituents and antioxidant activity of *Alstonia boonei. African Journal of Biotechnology*. 2007, 6 (10), 1197-1201

[36]. Arulmozhi, S., Mazumder, P.M., Ashok, P., Narayanan, L. S. Anti-nociceptive and anti-inflammatory activities of *Alstonia scholaris* Linn. R.Br. *Pharmacognosy Magazine*. 2007, *3*(10), 106-111.

[37]. Arulmozhi, S., Rasal, V.P., Narayanan, L. S., Ashok, P. Screening of *Alstonia scholaris* Linn. R.Br., for wound healing activity. *Oriental Pharmacy and Experimental Medicine*. 2007, 7 (3) (article in press).

[38]. Lin, S.C., Lin, C.C., Lin, Y.H., Supriyatna, S., and Pan, S.L. The protective effect of *Alstonia scholaris R.Br.* on hepatotoxin-induced acute liver damage. *Am. J. Clin. Med*. 1996, *24*, 2, 153-64.

[39]. Goyal, M.M. and Varshney, A. Effects of natural products isolated from three species of *Alstonia* on some gram-positive and gram-negative bacteria. *Indian Drugs*. 1995, *32*, 2, 69-72.

[40]. Khan, M..R., Omoloso, A.D., Kihara, M. Antibacterial activity of *Alstonia scholaris* and *Leea tetramera. Fitoterapia*. 2003, *74*, 736–740.

[41]. Khyade, M. S., Vaikos, N. P. Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. *African Journal of Biotechnology*. 2009, *8*(22), 6434-6436.

[42]. Thankamani, V., James, J., Veettil, A.K.T. and Sagadevan, L.D.M. Phytochemical screening and anti microbial activity of *Alstonia scholaris* flowers (L) R.BR. Fam: Apocynaceae. *International Journal of Pharmaceutical Research and Development* 2011; *3*(3): 172-178.

[43]. Misra, C.S., Pratyush, K., Sagadevan, L.D.M., James, J., Veettil, A.K.T. and Thankamani, V. A comparative study on phytochemical screening and antibacterial activity of roots of *Alstonia scholaris* with the roots, leaves and stem bark. *International Journal of Research in Phytochemistry and Pharmacology* 2011; *1*(2):77-82.

[44]. Amole, O.O. and Ilori, O.O. Antimicrobial Activity Of The Aqueous And Ethanolic Extracts Of The Stem Bark Of *Alstonia Boonei. International Journal of Phytopharmacology*. 2010, *1*(2), 119-123.

[45]. Javaid, A., Shafique, S., Bajwa, R. and Shafique, S. Parthenium Management Through Aqueous Extracts Of *Alstonia scholaris. Pak. J. Bot*. 2010, *42*(5), 3651-3657.

[46]. Lee, S.J., Cho, S.A., An. S.S., Na, Y.J., Park. N.H., Kim, H.S., Lee, C.W., Kim, H. K., Kim, E.K., Jang, Y.P., Kim, J.W. *Alstonia scholaris* R. Br. Significantly Inhibits Retinoid-Induced Skin Irritation *In Vitro* and *In Vivo.*

[47]. Thankamani, V., Pratyush, K., Misra, C.S., James, J., Sagadevan, L.D.M., Veettil, A.K.T. FT-IR Analysis And In Vitro Cytotoxicity Assay Of Methanolic Extract Of Roots Of *Alstonia scholaris. International Journal of Institutional Pharmacy and Life Sciences*. 2011, *1*(1), 53-67.

[48]. Shah, A.J., Gowani, S.A., Zuberi, A.J., Ghayur, M.N., Gilani, A.H. Antidiarrhoel and spasmolytic activities of the methanolic crude extract of *Alstonia scholaris* L. are mediated through calcium channel blockade. *Phytotherapy Research*. 2010, *24*, 1, 28-32.

[49]. Shang, J. H., Cai, X.H., Zhao, Y.L., Feng, T., Luo, X. D. Pharmacological evaluation of *Alstonia scholaris*: Anti-tussive, anti-asthmatic and expectorant activities. *Journal of Ethnopharmacology*. 2010, *129*, 293-298.

[50]. Ogbonnia, S., Adekunle, A. A., Bosa, M. K. and Enwuru, V. N. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *African Journal of Biotechnology*. 2008, *7* (6), 701-705.

[51]. Jagetia, G.C. and Baliga, M.S. Induction of developmental toxicity in mice treated with *Alstonia scholaris* (Sapthaparna) In utero. *Repro Toxicol*. 2003, *68*, 6, 472-478.