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EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF *ALSTONIA SCHOLARIS* STEM BARK AGAINST CCL4 INDUCED HEPATOTOXICITY – AN *IN-VIVO* SCREENING

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ABSTRACT: The hepatoprotective potential of ethanolic extract of stem bark of *Alstonia scholaris* belonging to the family Apocynaceae and commonly known as Devil's tree or Saptaparni was assessed against carbon tetrachloride-induced hepatotoxicity in Wistar rats. It is an evergreen tropical tree native to the Indian sub-continent and Southeast Asia and contains various phytoconstituents like alkaloids, triterpenoids, flavonoids, steroids, and phenolic acids which have shown promising therapeutic potential. The hepatoprotective effect was assessed by evaluating the biochemical parameters like serum alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and total bilirubin and histopathological studies of the liver. Rats treated with ethanolic extracts of *Alstonia scholaris* in the dose of 150 mg/kg and 300 mg/kg, evinced a considerable reduction in CCl_4 induced augmented serum enzyme levels (ALT, AST, ALP, and total bilirubin) and also a considerable increase in total protein levels that was lowered by hepatotoxic compound used, comparable with Silymarin (standard drug). *Alstonia scholaris* was also able to significantly prevent the rise in MDA level as evident by the TBARS test for *in-vivo* lipid peroxidation. Histopathology of the extract-treated groups showed a lessening of the pathogenesis and revealed a marked reduction in hepatic injuries that was equivalent to the Silymarin-treated group.

INTRODUCTION: Liver is the largest internal organ and also the largest gland of the body that is found in the upper right-hand part of the abdominal sac, below the diaphragm and on top of the stomach, right kidney and the intestines. Weighing about three pounds, it has a cone-like structure that is soft and pinkish-brown in colour [1].

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It consists of two main lobes- the left and the right lobes, consisting of 8-sections containing thousands of lobules. These lobules join with tiny ducts that further attach with bigger ducts forming a common hepatic duct that transports the bile juices made by the liver cells to the gallbladder and the duodenum through a common bile duct. Almost 13% of the body's blood flows through the liver at any period of time [2].

"Hepatocytes- the regenerative cells" form the main tissue cells of the liver that constitute around 70-80% of the cytoplasmic mass. They are polygonal cells having a single round nucleus and a distinguished nucleolus [3].

The liver performs dual roles as a metabolic and biochemical transformation factory. The portal vein receives blood containing substances, and *via* the hepatic artery, oxygen-rich blood is received [4]. Thus a liver is classified as a "bidirectional biofilter" [5] which performs a vast variety of functions.

As the liver functions' in eliminating substances via the portal circulation, thus it becomes prone to relentless assault by external compounds, leading to hepatic disorders [6]. Liver disorders stay one of the significant dangers to general wellbeing and thus pose serious health issues worldwide [7].

Under experimental conditions, hepatic injury can be caused either by partial hepatectomy or by various hepatotoxins like carbon-tetrachloride (CCl_4), thioacetamide, D-galactosamine, chloroform, paracetamol, arsenic, ethyl alcohol and pyridine [8].

Among many natural products, plants serve as a major chemical substance source by acting either directly as drugs or as a key component in synthetic drug formulations. For a successful examination, the right choice of plant species plays an important role. Though arbitrary choice gives a few hints, directed assortment dependent on chemotaxonomic connections and ethnomedical data inferred from traditional medication are bound to yield pharmacologically active constituents [9, 10]. The use of herbal drugs in treating liver diseases has long folklore, but proof of its efficacy is inadequate. Additionally, there are worries about the nature of studies testing herbal medicines. Notwithstanding these constraints, various herbal drugs show promising impacts, tentatively in cell culture, animal studies, or clinical preliminaries. Hepatoprotective plants contain chemical components like phenols, coumarins, monoterpenes, glycosides, alkaloids, and xanthenes [11].

One such plant is *Alstonia scholaris* (family Apocynaceae), popularly known as "Devil's tree" or "Saptaparni" and is attaining the attention of researcher's for its pharmacological activities. Various phytoconstituents have been reported in different parts of the plant, such as bark [12], leaves [13], roots [14], flowers and fruits [16] such as alkaloids, iridoids, coumarins, flavonoids, leucoanthocyanins, reducing sugars, simple phenolics, steroids, saponins and tannins [17]. It has been reported to possess antimalarial [18], antimicrobial [19], free radical scavenging and antioxidant [20], anti-diabetic [21], analgesic and anti-inflammatory [22], anticancer and cytotoxicity [23, 24], radioprotective [25], CNS activity [26], immunostimulating [27], antifertility [28], antidiarrheal [29], bronchodilatory [30], anti-tussive and anti-asthmatic [31] activities. Keeping in view the biologically active nature of *A. scholaris*, the current study aims to assess the hepatoprotective potential of stem bark of *A. scholaris* against carbon tetrachloride-induced hepatic injury.

MATERIALS AND METHODS:

Chemicals: All the chemicals and solvents utilized in the study were of analytical grade and procured from the local market of Ghaziabad. The assay kits utilized for the estimation of biochemical parameters like ALP, AST, ALT, total bilirubin, total protein, *etc*., were acquired from ERBA (Himachal Pradesh, India).

Plant Collection and Authentication: The mature stem bark from the woody trunk portion of *Alstonia scholaris* was procured from the local area of Ghaziabad, Uttar Pradesh, India. It was authenticated by an emeritus scientist, Dr. Sunita Garg of Raw Material Herbarium and Museum (RHMD), Delhi (taxonomic reference number: NISCAIR/RHMD-3653-54).

Preparation of the Extract: Shade dried stem bark was grounded into fine powder with the help of an electric grinder and was subjected to continuous extraction with hot ethanol using Soxhlet apparatus [32, 33]. The extract so obtained was filtered, and the filtrate was made solvent-free using a rotary vacuum evaporator and was subjected to preliminary phytochemical analysis [34].

***In-vitro* Antioxidant Activity:** The free radical scavenging activity of ethanolic extract of *Alstonia scholaris* (EEAS) stem bark was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) against standard ascorbic acid, utilizing the standard method [35]. Concisely, the reaction mixture contained various concentrations of the ethanolic extract and DPPH in ethanol. The mixture was shaken, left for 25-30 min at room temperature in the dark, and the

reduction of DPPH radical was determined by reading the decrease in absorbance at 517nm using a UV-visible spectrophotometer against the corresponding blank (ethanol). Ascorbic acid was used as a reference compound. All determinations were performed in triplicate [36, 37]. The results revealed that EEAS showed the highest antioxidant activity of 80.3% as compared to the standard ascorbic acid. Thus EEAS has proton donating ability and could serve as a free radical inhibitor or scavenger and act as a natural antioxidant.

***In-vivo* Activity:** Wistar Rats (either sex), weighing between 300-350 g, were procured from the animal house of Meerut Institute of Engineering And Technology, Meerut, Uttar Pradesh, India. The animals were quarantined and housed in an Animal House Facility for acclimatization for seven days prior to experimentation. Animals were housed in polypropylene cages with dust-free rice husk as a bedding material and maintained under standard laboratory conditions with controlled temperature (23 ± 2°C), humidity (40 ± 10%), and a natural (12 hour each) light-dark cycle. They were fed with a standard rodent pellet diet and water *ad libitum.* The care of laboratory animals was done as per the guidelines of CPCSEA, Ministry of Forests & Environment, Government of India. The research protocol of this study was approved by the IAEC of Meerut Institute of Engineering And Technology (registration no. IAEC/MIET/2021/27).

Experimental Design:

Acute Toxicological Studies: Wistar rats (n=3) were selected by random sampling technique, and OECD-423 guidelines [38] were followed to study the acute oral toxicity. The ethanolic extract was administered orally, starting with 5 mg/kg body weight up to 2 g/kg b.w. Initially, for the first hour after administering the dose, individual animals were monitored at least once and then regularly throughout the first 24 h, with specific care being given during the first 4 h, every day after that for a total of 14 days.

Hepatoprotective Activity against Carbon Tetrachloride Induced Hepatotoxicity: Hepatotoxicity was induced in rats by intraperitoneal administration of carbon tetrachloride (CCl_4), and Silymarin was used as a reference standard. To study the activity, animals were randomly divided into five groups (n=6 animals in each group). The first group served as normal control and received normal saline only (10 mL/kg of b.w.). The second group served as a disease control group and received CCl_4 dissolved in olive oil (3 mL/kg of b.w.) i.p., twice a week for 4 weeks. The third group was pre-administered with CCl_4 as in group 2 followed by Silymarin (100 mg/kg *p.o.*) daily for 4 weeks. Groups 4 and 5 received an ethanolic extract of stem bark of *Alstonia scholaris* in the dose of 150 mg/kg and 300 mg/kg, respectively, daily and 30 min before the administration of carbon tetrachloride, on the days of CCl_4 administration (as in group 2), for 4 weeks. After twenty-four hours of the last dose, the animals were euthanized and liver and blood samples were collected for the biochemical estimations and histopathological evaluations.

Assessment of Liver Function: The biochemical investigation for assessing liver functions was done where estimation of serum enzymes like ALT, AST [39] and ALP [40], total bilirubin, total proteins [41, 42], serum albumin [43] and TBARS [44, 45] was carried out to study the effect of ethanolic extracts on liver toxicity induced by CCl_4.

Histopathological Analysis: The liver tissues preserved in 10% formalin were dehydrated in graded concentrations of ethanol, immersed in xylene, and then embedded in paraffin. The sections of 4 µm thickness were cut and stained with haematoxylin and eosin [46]. The slides were observed for gross histopathological changes and neutrophil accumulation.

Statistical Analysis: All obtained values were expressed as mean ± standard error mean (SEM). The significance of the difference was statistically analyzed using a one-way analysis of variance (ANOVA) and $P<0.05$ was considered statistically significant. The statistical analysis of the data was performed using GraphPad Prism 5 software (GraphPad Inc., La Jolla CA).

RESULTS AND DISCUSSION: Numerous herbs are said to offer alleviation of hepatic diseases in the Indian medical system. The stated therapeutic reputation of such herbs must be scientifically confirmed. Thus in the current research work, the ethanolic extract of *Alstonia scholaris* stem bark

was investigated for its hepatoprotective study. The percentage yield obtained from the ethanolic extract was found to be 13.9 %, and the preliminary phytochemical evaluation revealed the presence of plant secondary metabolites such as alkaloids, flavonoids, saponins, and carbohydrates, terpenoids and tannins in the ethanolic extract of *Alstonia scholaris* stem bark **Table 1.**

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *ALSTONIA SCHOLARIS* STEMBARK

Phytochemical Group	EEAS
Flavonoids	+
Phenols	-
Alkaloids	+
Tannins	-
Steroids and sterols	-
Terpenoids	+
Glycosides	+
Saponins	+
Carbohydrates	+
Amino acids and proteins	-

+ = Presence of active constituents - = Absence of active constituents.

As the ethanolic extract showed the presence of maximum number of phytoconstituents, thus it was evaluated for hepatoprotective potential. The ethanolic extract of stem bark of *Alstonia scholaris* was found to be nontoxic up to a dose of 2 g/kg with no signs of mortality in test animals. A best known example of a zone-3 hepatotoxin is carbon tetrachloride which causes necrosis and liver failure. Its metabolism produces a hazardous trichloromethyl radical, which causes injury.

In the present work, it was perceived that the rats treated with carbon tetrachloride developed significant hepatic damage as seen by augmented serum levels of hepato-specific enzymes like ALT (SGPT), AST (SGOT), ALP and total bilirubin and lowered serum total protein levels when compared to normal control. Pre-treatment with Silymarin and EEAS showed good protection against carbon tetrachloride-induced liver toxicity. Tests indicate a significant reduction in augmented serum enzyme levels and a considerable increase in total protein levels with extract-treated animals, evident from **Table 2.**

In-vivo lipid peroxidation study (TBARS) revealed that the CCl_4 treated group showed a significant increase in malondialdehyde (MDA) level when compared with the normal control group. EEAS was able to significantly prevent this rise in MDA level, as evident from **Table 2.**

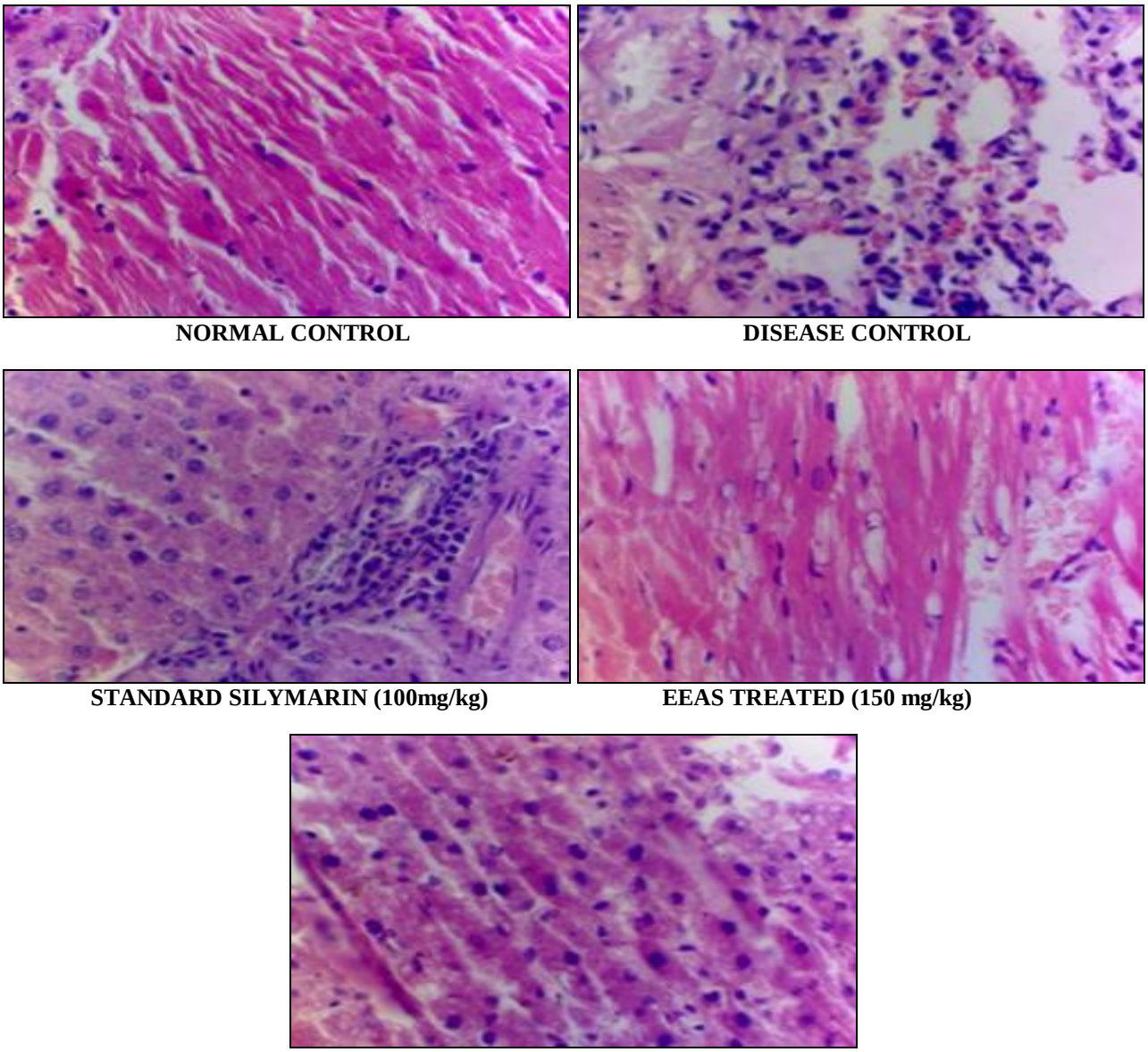
TABLE 2: EFFECT OF ETHANOLIC EXTRACTS OF STEM BARK OF *ALSTONIA SCHOLARIS* (EEAS) ON ALT, AST, ALP, TOTAL BILIRUBIN, TOTAL PROTEINS, SERUM ALBUMIN, AND LPO LEVELS IN CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

Group	Serum ALT Levels (IU/L)	Serum AST Levels (IU/L)	Serum ALP Levels (IU/L)	Total bilirubin Levels (mg/dl)	Total protein levels (g/dl)	Serum albumin (g/dl)	LPO (n Mole of MDA/mg of protein)
1	39.88±5.52	41.77± 5.42	101.66±11.88	0.72± 0.09	7.59± 0.86	4.61 ±0.53	0.78 ±0.08
2	82.67±10.67	91.35± 10.25	226.91±24.34	1.81± 0.19	3.06± 0.41	1.51 ±0.18	2.63 ±0.27
3	51.88±4.96***	61.62±6.40***	127.54±13.45***	1.01± 0.14**	5.58± 0.3***	3.68 ±0.40***	1.55± 0.18**
4	65.46±6.77*	71..88±8.750*	174.36±18.62*	1.33± 0.16*	4.64± 0.485**	2.66 ±0.27*	2.22±0.26*
5	42.8±4.71***	52.73±5.90***	122.19±13.22***	0.88± 0.091***	6.39± 0.59**	3.97±0.42***	1.2± 0.15***

Values are mean ± SEM (n=6) one-way ANOVA. Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$. All values are compared with the toxicant.

Histopathological Studies of the Liver in Carbon Tetrachloride Induced Hepatotoxicity: The histopathological evaluation of carbon tetrachloride toxicity in all the groups was examined and shown in **Fig. 1**. The rat liver section treated with the normal control group shows liver parenchyma with intact architecture, which is the usual morphology, whereas the architecture of the liver in the disease control group is partially effaced; certain hepatocytes show apoptotic changes, perivenular mononuclear inflammatory infiltration, and scattered inflammatory infiltration within the

parenchyma as signs of toxicity. In contrast, the liver section in the Silymarin treated group shows liver parenchyma with intact architecture though some of the central veins show congestion with diffuse sinusoids. Also, the liver sections taken from EEAS treated groups (150 and 300 mg/kg) show lessening of the pathogenesis and revealed a marked reduction in hepatic injuries that is equivalent to Silymarin treated group. Pursuant to these findings, flavonoids and saponins found in EEAS may be responsible for the hepatoprotective action. It is clear from the findings of this study that compared to Silymarin, the ethanolic extract of *Alstonia scholaris* stem bark also offers a pharmacologically fruitful treatment for a variety of liver ailments, indicating an improvement in the liver's functional status, which was also supported by histopathological findings.



NORMAL CONTROL **DISEASE CONTROL**

STANDARD SILYMARIN (100mg/kg) **EEAS TREATED (150 mg/kg)**

EEAS TREATED (300 mg/kg)

FIG. 1: HISTOPATHOLOGY OF THE RAT LIVER IN CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY

CONCLUSION: The studies above reveal that treatment with ethanolic extract of *A. scholaris* stem bark was efficacious in offering protection against hepatotoxicity caused by carbon tetrachloride. The hepatoprotective potential of 300 mg/kg was substantially greater compared to 150 mg/kg. Histological observations corroborated the extract's therapeutic properties.

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REFERENCE:

1. Searle C: The liver: the great purifying organ of the body; its importance to health and the extreme frequency of its disorders. Read Books Publisher London 2009.
2. Hoffman M: Human Anatomy- Picture of the Liver. Reviewed by Carol DerSarkissian 2019. https://www.webmd.com/digestive-disorders/picture-of-the-liver.
3. Mohan H: Textbook of Pathology. Jaypee Brothers Medical Publishers, India, Eighth Edition 2018; 592-648.
4. Muriel P: Liver Pathophysiology- Therapies and Antioxidants. Academic Press, Elsevier London 2017.
5. Arias IM, Alter HJ, Boyer JL, Cohen DE, Shafritz DA, Thorgeirsson SS and Wolkoff AW: The Liver-biology and Pathobiology. Hoboken NJ: Wiley-Blackwell, Sixth Edition 2020.
6. Bodakhe SH and Ram A: Hepatoprotective Properties of Bauhinia variegataBark extract. Yakugaku Zasshi 2007; 127: 1503-7.
7. Eswar KA, Susmitha K, Swathy B, Ramu E and Venkatesh B: A review on liver disorders and screening models of hepatoprotective agents. International Journal of Allied Medical Sciences and Clinical Research 2014; 2(2): 136-50.
8. Pizzorno JE and Murray MT: Textbook of Natural Medicine. Churchill Livingstone Fifth Edition 2020.
9. Mohiuddin AK: A Brief review of traditional plants as sources of pharmacological interests. Open Journal of Plant Science 2019; 4(1): 1-8.
10. Koparde AA, Doijad RC and Magdum CS: Natural Products in Drug Discovery. Pharmacognosy - Medicinal Plants, edited by Shagufta Perveen, Areej Al-Taweel, Intech Open London 2019.
11. Ilyas U, Katare DP, Aeri V and Naseef PP: A Review on hepatoprotective and immunomodulatory herbal plants. Pharmacognosy Review 2016; 10(19): 66-70.
12. Bhawna S and Kumar SU: Hepatoprotective activity of some indigenous plants. International Journal of Pharm Tech Research 2009; 4: 1330-4.
13. Feng T, Cai XH, Zhao PJ, Du ZZ, Li WQ and Luo XD:Monoterpenoidindole alkaloids from the bark of Alstoniascholaris. Planta Medica 2009; 75(14): 1537-41.
14. Rahman AU, Alvi KA and Muzaffar A: Isolation and 1H/13C NMR Studies on 19, 20-Dihydrocondylocarpine: An alkaloid from the leaves of Ervatamiacoronaria and Alstonia scholaris. Planta Medica 1986; (4): 325-6.
15. Boonchuay W and Court WE: Minor alkaloids of *Alstonia scholaris* Root. Phytochemistry 1976; 15.
16. Dutta SC, Bhattacharya SK and Ray AB: Flower alkaloids of *Alstonia scholaris*. Planta Medica 1976; 30(1): 86-90.
17. Wongseripipatana S, Chaisri L, Sritularak B and Likhitwitayawuid K: Indole alkaloids from the fruits of Alstonia scholaris. Thai Journal of Pharmaceutical Sciences 2004; 28: 173-80.
18. Khyade MS and Vaikos NP: Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br. African Journal of Biotechnology 2009; 8(22): 6434-6.
19. Gandhi M and Vinayak VK: Preliminary evaluation of extracts of *Alstonia scholaris* bark for *in-vivo* antimalarial activity in mice. Journal of Ethnopharmacology 1990; 29(1): 51-7.
20. Singh B and Sangwan P: Taxonomy, ethnobotany and antimicrobial activity of *Alstonia scholaris* (L.) R. Br., *Carissa carandas* L. and *Catharan thusroseus* (L.) G. Don. International Journal of Biotechnology and Biosciences 2011; 1(1): 102-12.
21. Arulmozhi S, Mazumder PM, Ashok P and Narayanan LS: *In-vitro* Anti- oxidant and free radical scavenging activity of *Alstonia scholaris* Linn. R. Br. Iranian Journal of Pharmacology and Therapeutics 2007; 6(2): 191-6.
22. Bandawane D, Juvekar A and Juvekar M: Anti-diabetic and anti-hyperlipidemic effect of *Alstonia scholaris* Linn. bark in streptozotocin induced diabetic rats. Indian Journal of Pharmaceutical Education and Research 2011; 45(2): 114-20.
23. Arulmozhi S, Mazumder PM, Sathiyanarayanan L and Thakurdesai PA: Analgesic, anti-inflammatory and anti-ulcerogenic activities of fractions from *Alstonia scholaris*. Pharmacologia 2012; 3(5): 132-7.
24. Jagetia GC and Baliga MS: Evaluation of anti-cancer activity of the alkaloid fraction of *Alstonia scholaris* (Sapthaparna) *in-vitro* and *in-vivo*. Phytotherapy Research 2006; 20(2): 103-9.
25. Sharma V, Mallick SA and Tiku AK: Anticancer activity of Devil tree (*Alstonia scholaris* Linn.) leaves on Human cancer cell lines. Indian Journal of Agricultural Biochemistry 2010; 23(1): 63-5.
26. Gupta U, Chaudhary R and Goyal PK: Post-treatment effects of *Alstonia scholaris* extract against radiation-induced biochemical alterations in Swiss albino mice. Iranian Journal of Radiation Research 2010; 8(3): 169-77.
27. Kulkarni MP and Juvekar AR: Effect of *Alstonia scholaris* (Linn.) R. Br. on stress and cognition in mice. Indian Journal of Experimental Biology 2009; 47(1): 47-52.
28. Iwo MI, Soemardji AA, Retnoningrum DS and Sukrasno UM: Immuno stimulating effect of pule (*Alstonia scholaris* L.R. Br., Apocynaceae) bark extracts. Clinical Hemorheol and Microcirculation 2000; 23(2-4): 177-83.
29. Gupta RS, Sharma R, Sharma A, Bhatnager AK, Dobhal MP, Joshi YC and Sharma MC: Effect of *Alstonia scholaris* bark extract on testicular function of Wistar rats. Asian Journal of Andrology 2002; 4(3): 175-8.
30. Patil S, Juvekar AR, Joglekar SN, Shamkuwar PB and Nimbkar SR: Study of antidiarrhoeal activity of *Alstonia scholaris* bark. Indian Drugs 1999; 36: 463-5.
31. Channa S, Dar A, Ahmed S and Atta-ur-Rahman: Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. J of Ethnophar 2005; 97(3): 469-76.
32. Zygler A, Słomińska M and Namieśnik J: Soxhlet Extraction and New Developments Such as Soxtec-Comprehensive Sampling and Sample Preparation. Academic Press 2012: 65-82.
33. Harborne JB: Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd, 1973.
34. Bulugahapitiya VP: Plants Based Natural products: Extraction, Isolation and Phytochemical screening methods. First Edition 2013.
35. Vinodhini S, Preethi M, Fathima NN, Kushwaha SS and Rajeswari DV: Antioxidant and Free Radical Scavenging Capacity of Extensively Used Medicinal Plant of *Punica granatum*. Asian Journal of Pharmaceutical and Clinical Research 2016; 9(6): 140-6.

36. Blois MS: Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199–2000.
37. Rahman MM, Islam MB, Biswas, M and Alam AHMK: *In-vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Res Notes 8 2015; 621.
38. Sodhi JK, Kaur A, Shrivastava B and Lamba HS and Sharma J: *In-vitro* antioxidant activity of ethanolic extract of bark of *Alstonia scholaris*. International Journal of Psychosocial Rehabilitation 2020; 24(04): 10502-9.
39. OECD. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, 2002. https://doi.org/10.1787/9789264071001-en.
40. Reitman S and Frankel S: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 1957; 28(1): 56-63.
41. Burtis CA, Ashwood ER and Bruns DE: Textbook of Clinical Chemistry and Molecular Diagnostics. WB Saunders Comp Fifth Edition 2012.
42. Doumas BT: Standards for Total Serum Protein Assays- A Collaborative Study. Clinical Chemistry 1975; 21(8): 1159-66.
43. Tietz NW: Textbook of Clinical Chemistry. WB Saunders Philadelphia 1986; 579.
44. Doumas BT, Arends RL and Pinto PC: Standard Methods of Clinical Chemistry. Academic Chicago Press 1972; 175-89.
45. Wills ED: Lipid peroxide formation in microsomes. General considerations. Biochem J 1969; 113(2): 315-24.
46. Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979; 95: 351-8.
47. Clayden EC: Practical Section Cutting and Staining. Churchill Livingstone: Edinburgh, Fifth Edition 1971.

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