International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 01-11-2021, Accepted: 17-11-2021, Published: 02-12-2021
Volume 6, Issue 6, 2021, Page No. 630-633

Extraction, phytochemical screening and acute toxicity studies of *Abutilon indicum*, *Phyllanthus niruri*, *Eclipta alba* and *Allium sativa*

Deepika Patel*, Mohit Chaturvedi
Faculty of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Abstract
Abutilon indicum (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) are well known medicinal plants as mentioned in Ayurveda and folk remedies. In the present paper extraction, phytochemical screening and acute toxicity studies of aqueous extract were mentioned. The results indicate that aqueous extract were found to be safe for use.

Keywords: medicinal plants, extract, acute toxicity studies

Introduction

Indian Indigenous medicinal plants are most widely used for the treatment of several diseases either in alone or in combination in raw as well as their extract. Ancient ayurvedic literature reveals that the selected plants i.e., Abutilon indicum (Leaves), *Phyllanthus niruri* (Fruits), *Eclipta alba* (Leaves) and *Allium sativa* (Bulb) have been widely used in the treatment of liver disorders.

Abutilon indicum (Linn.) Sweet. (Kanghi), Family Malvaceae, Found in wild state in Central India. The plant contains saponins, flavonoids, alkaloids. The important constituents reported in the plant are β-sitosterol, vanillic acid, p-coumaric acid, caffeic acid, fumaric acid. The leaves of the plant contain steroids, sapogenins, carbohydrates and flavonoids. Almost all the parts are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, in chest infection and urethritis. The infusion of the root is prescribed in fevers as a cooling medicine and is considered useful in strangury, haematuria and in leprosy. The leaves are found to be good for ulcer and to treat liver disorders. The bark is used as febrifuge, anthelmintic, alexeteric, astringent and diuretic. The seeds are used in piles, laxative, expectorant, in chronic cystitis, gleet and gonorrhea.

Phyllanthus niruri *Linn*. (Blue amala) Family: Phyllanthaceae, Found in India in rainy to winter seasonThe plant contains different classes of organic compounds. Major constituents are lignans, tannins, polyphenols, alkaloids, flavonoids, terpenoids and steroid. It has extensive medicinal properties and has long history in the health care system of tropical countries. The plant is used as a folk medicine for treating kidney stones, gallbladder stones, liver related diseases such as liver cancer & jaundice, apart from these it is also administered for diuretic, hypoglycemic and hypertension cases and it also shows anti in flammatory, antitumor, anti nociceptive and antioxidant properties.

Eclipta alba Linn. (Bhringraj) Family: Asteraceae**,** Found in wild state all over India. The plant contains wide range of active principles which includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids. The leaves contain stigmasterol, β-terthienylmethanol, wedelolactone, demethyl wedelolactone and demethylwedelolactone-7-glucoside. It has been as an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases. The plant is commonly used in hair oil all over India for healthy black and long hair. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and popularly used to enhance memory and learning.

Allium sativa Linn. (Garlic) Family: Liliaceae**,** Cultivated all over India for its bulb**.** It contains 33 sulphur compound, 17 amino acids, several enzymes and minerals such as selenium etc. Also, contains higher concentration of sulphur compounds than other Allium species. The sulphur compounds are responsible for its pungent odour and therapeutic action. Dried powdered garlic contain at least 1% alliin (S-allyl cysteine sulfoxide). Traditionally in Ayurveda, it has used for promotion of health as well as for the management of wide range of disorders including skin disorders, impaired digestive power, anorexia, indigestion, constipation, as analgesic, worm infestation, chronic cough, asthma and liver disorders [1-4].

Material and Methods

Extraction of selected herbs

The shade dried coarsely powdered plant material (250 gms) of plant *viz*., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were loaded in Soxhlet apparatus and was extracted with water for 48 hour. After completion of extraction, the solvent was removed by evaporation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined [5-6].

Preliminary phytochemical screening of extracts

The aqueous extract of plant *viz*., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba*

(Leaves) and AS: *Allium sativa* (Bulb) obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedures were adopted to perform the study [7-8].

Test for alkaloids
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml dilute sulphuric acid and were filtered. The filtrate (about 1 ml) thus obtained was tested with mayer's reagent, dragendroff's reagent, hager's reagent and wagner's reagents respectively. Appearance of cream, orange brown, yellow and reddish brown precipitate indicates the positive results and finally the presence of alkaloids.

Test for carbohydrates
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml of water and were filtered. The filtrate (about 1 ml) thus obtained was treated with 1ml concentrated sulphuric acid followed by 1 ml molisch's reagent. Appearance of pink to violet color confirms the presence of carbohydrates.

Test for tannins
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml water and were treated with 1 ml of 5% ferric chloride solution. Appearance of blue color indicates the presence of tannins.

Test for steroids and triterpenoids
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were refluxed with 10 ml alcoholic potassium hydroxide until the saponification takes place and the mixture was diluted with distilled water and ether (1:1), the extract was evaporated and subjected to Liebermann burchad's test. Brown ring which turns green confirm presence of steroids and deep red color indicate presence of triterpenoids.

Test for glycosides
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml of water and treated with brontrager's test. Pink color indicates confirm presence of glycosides.

Test for gums and mucilage
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml alcohol with constant stirring, formation of precipitate indicates presence of gums and mucilages.

Test for fixed oil and fats
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 1 ml of alcohol and was pressed between two filter paper, presence of oil stain in paper indicates presence of fixed oil and fats.

Test for saponins
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml of water and was shaken to produce the foam, formation of stable foam indicates presence of saponins.

Test for flavonoids
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml of alcohol and subjected to shinoda test, appearance of pink color indicates presence of flavonoids.

Test for proteins and amino acid
The aqueous extracts (about 1 mg) of dried plant material of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were dissolved in 10 ml of alcohol and subjected to 1 ml of ninhydrin reagent, purple color indicate presence of proteins and amino acid.

Acute toxicity studies of extracts
Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. [12].
Following are the main type of guideline followed by OECD

- Guideline 420, Fixed dose procedure. (5 animals used)
- Guideline 423, Acute toxic class. (3 animals used)
- Guideline 425, Up and down method. (1 animal used)

Procurement of experimental animals
The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment [9]. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization. IEAC approval no. IAEC/2018-19/RP-004 DATED 04/4/2019.

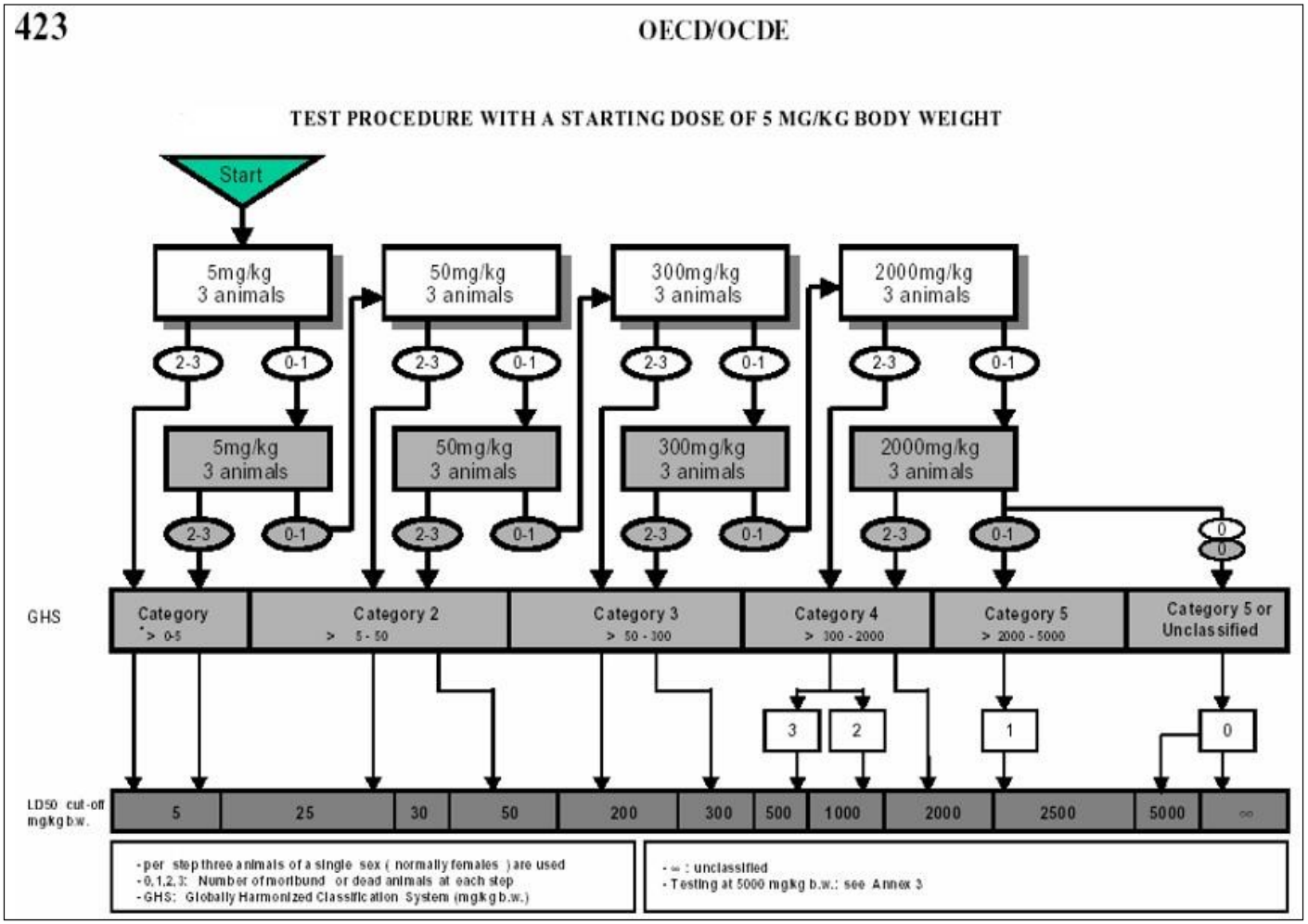


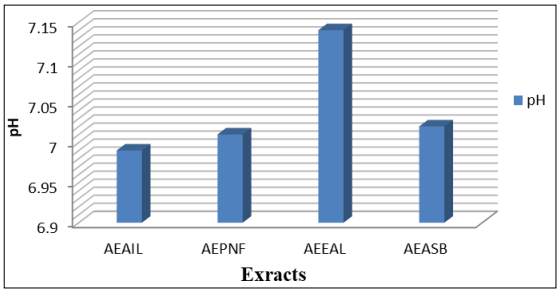
Fig 1: Calculation of dose as per OECD

Results and Discussion

The shade dried coarsely powdered plant material of AI: A*butilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) and was extracted with water. The extracts obtained were evaluated for pH, color and % yield. The results are presented in table 1 and graph 2

Table 1: Estimation of % yield of various extract of selected plant material

S/no.	Extract	Parameters			
		Nature of extract	Color	pH	% Yield
1.	AEAIL	Solid Powder	Dark green	6.99	18.29
2.	AEPNF	Solid Powder	Brown green	7.01	14.91
3.	AEEAL	Solid Powder	Light green	7.14	10.43
4.	AEASB	Solid Powder	Creamish white	7.02	8.29



Graph 1: pH of extract

The aqueous extract of plant *viz*., AIL: A*butilon indicum* (Leaves), PNF: *Phyllanthus niruri* (Fruits), EAL: *Eclipta alba* (Leaves) and ASB: *Allium sativa* (Bulb) obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure was adopted to perform the study. The results are shown in table 2.

Table 2: Preliminary Phytochemical Screening of Aqueous Extract of Plant *viz*., AIL: A*butilon indicum* (Leaves), PNF: *Phyllanthus niruri* (Fruits), EAL: *Eclipta alba* (Leaves) and ASB: *Allium sativa* (Bulb)

S/No.	Constituents	Extract			
		AEAIL	AEPNF	AEEAL	AEASB
1.	Carbohydrates	+	+	+	+
2.	Glycosides	+	-	-	+
3.	Alkaloids	+	+	+	+
4.	Protein & Amino acid	-	-	+	+
5.	Tannins & Phenolic compounds	+	+	+	+
6.	Flavonoids	+	+	+	+
7.	Fixed oil and Fats	-	-	-	-
8.	Steriods & Triterpenoids	+	+	+	+
9.	Waxes	-	-	-	-
10.	Mucilage & Gums	-	-	-	-

The aqueous extracts of plant material i.e., AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were screened for acute toxicity study by OECD guideline no. 423 for determination of LD_{50}. The results showed that the aqueous extracts i.e., AEAIL, AEPNF, AEEAL and AEASB

were belonging to category-4. Hence, LD_{50} was 2000 mg/kg, therefore, ED_{50} was 200 mg/kg. Therefore, two doses of 100 and 200 mg were selected for present investigation. The results were presented in table 3.

Table 3: Determination of LD_{50} and ED_{50} of Aqueous Extract of AI: *Abutilon indicum* (Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb)

No. of animals	Extract dose (mg/kg)	No. of death of animals			
		AEAIL	AEPNF	AEEAL	AEASB
3	5	0	0	0	0
3	50	0	0	0	0
3	300	0	0	0	0
3	2000	0	0	0	0

Conclusion

The extraction of AI: Abutilon indicum(Leaves), PN: *Phyllanthus niruri* (Fruits), EA: *Eclipta alba* (Leaves) and AS: *Allium sativa* (Bulb) were carried using water and percentage of extract were reported, Also phytochemical screening was done to evaluate the active phytochemicals present in the aqueous extract. The acute toxicity using OECD guidelines were performed and it was found that the aqueous extract of the selected plant were safe.

References

1. Archna Sharma1, Sharma RA, Hemlata Singh. Phytochemical and Pharmacological Profile of Abutilon Indicum L. Sweet: A Review, Int. J. Pharm. Sci. Rev. Res,2013:20(1):120-127.
2. Narendra K, Swathi J, Sowjanya KM, Krishna Satya A. Phyllanthus niruri: A Review on its Ethno Botanical, Phytochemical and Pharmacological Profile, Journal of Pharmacy Research,2015:5(9):4681-4691.
3. Mithun NM, Shashidhara, Vivek Kumar R, Eclipta alba (L.). A Review on its Phytochemical and Pharmacological Profile, Pharmacologyonline,2011:1:345-357
4. Satyapal Sing, Rajendra Prasad, Rai NP. Phytochemical and pharmacological review on rasona (Allium sativum Linn.): a potentiaL Herb, International Journal of Phytopharmacy Research,2015:6(2):53-58.
5. Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, 1998, 8-30.
6. Divakar MC. Plant drug evaluation-a laboratory guide, published by, CD remedies, 2nd Ed, 2002, 84-92.
7. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, Delhi. 4th Edition, 1997, 107-111.
8. Harborne JB. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis, Chapman & Hall, London, UK, 3rd edition, 1998, 1-7.
9. OECD. Guidelines for the testing of chemicals revised draft guideline 423: Acute oral toxicity. France: Organization for Economic Cooperation and Development, 2000.
10. Ibrahim NA. An up-to-date review of digoxin toxicity and its management. Int J Res Pharm Pharm Sci. 2019;4(3):59-64.