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ANTI-MICROBIAL ACTIVITY OF *AERVA SANGUINOLENTA* (L.)

Madhumita Dey [1*], Goutam Mukhopadhyay [2]

[1]*GURUNANAK INSTITUTE OF PHARMACEUTICAL SCIENCE & TECHNOLOG 157/F, Nilgung Road, Panihati Kolkata- 700114. India.*

[2]*BCDA College of Pharmacy and Technology, Barasat, Kolkata, India.*

Abstract

Disease is the basic problem faced by humans too since prehistoric times. Nature has along with the diseases created their cure in the form of vegetables, minerals and animals [1].The relationship between man and plants has been close enough throughout the development of human culture. In the understanding of human diseases there has been continued interest in the drugs from the plant kingdom [2]. In the Vedic period, the Osadhisukta of the Rigveda is the oldest documented knowledge about plants and herbal medicines[3].The indigenous knowledge about plants and plant products is rather detailed and sophisticated and has evolved into a separate shashtra (branch of learning) itself, called Dravya-Guna Vijnyan Shashtra. The plant *Aerva sanguinolenta* (family Amaranthaceae) is a perennial herb. The vernacular name in Bengali of *Aerva sanguinolenta* is 'bishohari'. The plant is available in tropical countries of Asia, China, Malaysia and Indo-China regions. In China, it is known as Bai-huami, in Maharashtra as Burval, in Uttarakhand as Sufedphulia and in Assam as Soru-araksan.This work deals with *Aerva sanguinolenta,* of Amaranthaceae family. A very little pharmacological and phytochemical study has been performed on this plant. This gives an ample scope to study on the plant. The work has done on phytochemical evaluation and pharmacological evaluation to check antimicrobial activity of n-hexen and ethanolic extract of Aerva Sanguinolenta.

Keywords: *Aerva sanguinolenta,* Amaranthaceae, Antimicrobial activity.

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***Corresponding Author:** Madhumita Dey, *GURUNANAK INSTITUTE OF PHARMACEUTICAL SCIENCE & TECHNOLOGY. 157/F, Nilgung Road, Panihati Kolkata- 700114. India.* Email: dey.madhumita05@gmail.com

1. Introduction

From the ancient time mankind is using so many drugs from the plant, animal and mineral sources to treat various diseases and ailments, but he was not known which constituent is responsible for the medicinal activity. Probably all life forms are affected with disease. Disease is the basic problem faced by humans too since prehistoric times. Nature has along with the diseases created their cure in the form of vegetables, minerals and animals [1].The relationship between man and plants has been close enough throughout the development of human culture. In the understanding of human diseases there has been continued interest in the drugs from the plant kingdom [2]. Evidence for the existence of well-organised system of medicine in India can be traced back to the archaeological remains of Harappa and Mohenjo-Daro, from where even Silajit has been reported. Ayurveda is the oldest Indian indigenous medicine system, probably with its roots in the Indus Civilization. In the Vedic period, the Osadhisukta of the Rigveda is the oldest documented knowledge about plants and herbal medicines[3].The indigenous knowledge about plants and plant products is rather detailed and sophisticated and has evolved into a separate shashtra (branch of learning) itself, called Dravya-Guna Vijnyan Shashtra. The codified traditions have about 25,000 plant drugs formulations that have emerged from such studies. In addition to this over 50,000 formulations are believed to be existing in the folk and tribal traditions. All these point to the deep passion for an exhaustive knowledge about medicinal plants that have existed in the land from time immemorial. The Vedas, epic poems contain rich material on the Herbal role of that time [4].

THE INDIAN SYSTEM OF MEDICINE (ISM):

These are traditional system of medicine which encompasses 3 systems namely Ayurveda, Siddha and Unani, practiced by Vaidyas, Siddhas and Hakkims respectively. The medicines (or) formulations that come under Ayurveda, Siddha and Unani system of treatment are called as Indian System of Medicines. The Drug and Cosmetic Act defines the ISM as "Ayurvedic, Siddha and Unani drug includes all medicines, intended for internal or external use in the diagnosis, treatment, mitigation or prevention of diseases or disorder in human beings or animals"[5].

MODERN DRUGS FROM AYURVEDA:

Ayurveda the traditional Indian system of medicine is as old as the Indian culture and civilization. The earliest-recorded knowledge about Ayurveda is found in the *Rigveda* and the *Atharvaveda*, both of the second millennium BC. The *Atreya Samhita* is perhaps the oldest medical book in the world; it survives from Takshashila University, going back to the mid-I Millennium BC. The *Atharvaveda* lists eight divisions of Ayurveda: internal medicine, surgery of head and neck, ophthalmology, surgery, toxicology, psychiatry, pediatrics, gerontology or science of rejuvenation and the science of fertility. At about 500 BC in the University of Banaras, Sushrut, a surgeon, who developed the operative techniques of rhinoplasty (plastic surgery), wrote the *Sushruta Samhita*, which describes a highly developed surgery. The physician Charaka revised and supplemented the *Atreya Samhita*; in his book, the *Charak Samhita* is a vast work on internal medicine. More than 600 drugs of animal, plant and mineral origins are used in the Caraka and about 650 in the *Sushruta Samhita*[6].

Indian Ayurvedic system certainly has given birth to number of important and modern drugs, viz. ajmalicine, reserpine, leurocristine, L-dopa, cardiac glycosides, sennosides etc6.The importance

of plants as a source of useful antihypertensive drugs was supported by the isolation of reserpine from *Rauwolfia serpentina* by Muller et al in 1952. Veratrum alkaloids were other antihypertensive agents from plant sources[7].Number of medicinal plants has been reported to have anti-inflammatory activity; notable among these are Mesua ferrea, Azadirachta indica, Glycyrrhiza glabra, Cyperus rotandus, Curcuma longa [8].

FUTURE STRATEGIES/ PERSPECTIVES FOR INDIAN HERBAL MEDICINE:

Developing countries like India with traditional knowledge base have leadership potential to develop globally acceptable newer opportunities and applications for herbal industry. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness but they lack an experimental base and therefore have second class status whereas modern medicines have a very strong experimental basis for their use but have side effects. Thus, it seems, to get a new class of drugs, the researchers are increasingly blending the traditional knowledge with modern experimental methodology for testing the efficacy and safety of herbal drugs. This inclination seems to be resulted in the people all over the world looking to various alternative systems of medicine, especially herbal drugs which are claimed to be safe equally effective in comparison to allopathic drugs and which provide some answer to chronic diseases.[9] However, these herbal drugs are marketed with exaggerated claims or in some cases are credited with innumerable pharmacological activities which are not mentioned in the text of various traditional systems of medicine. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds [10]. A considerable amount of research on Pharmacognosy, Chemistry,Pharmacology and Clinical therapeutics has been carried out on Ayurvedic medicinal plants. The plants used in the Indian Systems of Medicine are of interest to find new leads for treating different diseases. Approaches like high-throughput screening, phytochemical profiling, quality control methods and standardization of raw materials and finished products, clinical trials, herbal therapeutics, pharmacokinetics and herbal pharmacovigilance will not only help to prove the rationale of using these systems but also to get maximum benefits of the natural resource [11].

Methods

PREPARATION AND EXTRACTION OF PLANT EXTRACT

CHEMICALS: n-hexane, ethyl Acetate, chloroform, Ethanol.

GLASS APPARATUS: Round bottomed flask, Sohxlet Apparatus, Measuring cylinder.

APPARATUS & INSTRUMENTS: Grinder, Sieve No. 40, heating mantle.

The aerial parts of the plant was shade dried. After proper drying, the size of the leaves was reduced with the help of grinder and it was passed through the sieve no 40. Different types of solvent are selected from low to high polarity for extraction. Temperature has to be maintained during soxhlation with the help of thermometer .Higher temperature may destroy the active chemical constitute of the plant. Distillation is performed to free the extract from solvent. The extracted product must be kept in a refrigerator.

Fig 1. Image of soxholator

PHYTOCHEMICAL STUDY OF *Aerva sanguinolenta*

Test for steroid

1.Salkowski test:

Procedure: - Few drops of extract is taken. Then add 2ml of chloroform and 2 to 3 drops of concentrated sulphuric acid and wait for some time.

Observation: - The color of chloroform layer show yellow color and the acid layer show yellow fluorescence.

Test for alkaloid

1. Wagner's test:

Procedure: - in this test, to the extract add 1.5ml of Wagner's reagent.

Observation: - precipitate is formed.

Test for glycosides

1. Procedure:-to the extract add potassium hydroxide solution.

Observation:-yellow green red color appeared.

2. Saponin glycoside test:

Procedure: - place 2ml solution of drug in water in that tube, shake well.

Observation: - stable fome formed.

3. Anthraquinone glycoside test (borntrager's test):

Procedure:-first boil the test material with 1ml of sulphuric acid in a test tube for five minutes. Then filter the hot solution through a filter paper. Then cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia.

Observation:-a rose pink to red color is produce in the ammoniacal layer.

Test for protein

1. Warming test:-

Procedure:-Heat the 1ml of test solution in boiling water bath.

Observation:-coagulation is form.

2. Biuret test:-

Procedure: - to the 2ml of test solution add 2ml of biuret reagent.

Observation: - volatile oil is appeared.

Test for mucilage

1. Test:

Procedure:-the extracted form of drug is examined under microscope.

Observation:-mucilage was found.

.**Test for flavonoids**

1.sinoda test:

Procedure: - first to the extract 5ml 95% ethanol is add. Then few drop of conc. hydrochloric acid is given. Now add 0.5mg of magnesium turning.

Observation:-pink color is arising.

a) Procedure: - to the small quantity of test residue, add lead acetate solution.

Observation:-yellow color precipitate

b) Procedure: - addition of increasing amount of sodium hydroxide to the residue.

Observation:-yellow color appears which discolor after addition of acid.

Test for tannin

1.test:

a) Procedure: - to 2 to 3 ml of aqueous extract add few drops of 5%ferric chloride.

Observation: - deep blue black color appears.

b) To 2 to 3ml of aqueous extract, add few drops of lead acetate solution.

Observation:-white precipitate is formed. [12]

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

Antimicrobial study is done by disc diffusion method.

DISC DUFFUSION METHOD

Isolation of microorganisms

From the soil sample of Guru Nanak Institute of Pharmaceutical Science & Technology, micro-organism was isolated by the technique of serial dilution.

Requirement:-

- Soil sample Molten and cooled.
- Nutrient agar media.

Composition of Nutrient Media

Peptone= 1gm

Beef extract= 0.6gm.

NaCl= 1gm

Agar= 3gm

Distilled water= 200ml

- Sterile water as blanks.
- Sterile 10-ml pipettes
- Magnetic stirrer
- Colony counter
- Bunsen burner
- Wax marking pencil.
- Sterile petridish

Procedure:-

1. Soil samples were collected at random, minimum five, from the field and mixed thoroughly to make a composite sample for microbiological analysis.

2. Dilution blanks were labeled as 1,2,3,4,5,6,7 and sterile Petri dishes as 10^{-2} (3plate), 10^{-3}(3plate), 10^{-4}(3plate),10^{-5}(3plate),10^{-6}(3plate) and 10^{-7}(3plate) with a wax pencil.

3. The initial dilution was prepared by adding 1mg of sample into a 9ml dilution blank labeled as 1 thus diluting the original sample 10 times(1/1+9 =1/10 and is written 1:10 or 10^{-1}

4. Mix the content by rolling the tube back and forth between hands to obtain uniform distribution of organisms (cells).
5. From the first dilution transfer 1ml of suspension to the dilution blank 2 with a fresh 1ml pipette diluting the original suspension to 100 times(1/10 *1/10 =1/100 or 10^{-2}).

6. From the 10^{-2} suspension transfer 1ml of suspension to 3 dilution blank with a pipette, thus the original sample to 1000 times(1:1000 or 10^{-3})
7. Repeat this procedure till the original sample has been diluted 10,000,000(10^{-7}) times using every time sterile pipette.

8. Transfer 1 ml adiquots each from 10-^{2} dilution blank into 3 sterile Petri dishes, from 10-^{3} dilution blank to 3 sterile Petri dishes, from 10^{-4} to 3 sterile Petri dishes, from 10^{-5} to 3 Petri dishes, from 10^{-6} to 3 Petri dishes and from 10^{-7} to 3 Petri dishes.

9. Add approximately 15 ml of the cooled medium (45°C) to each Petri dish and mix the inoculums by gentle rotation of the Petri dish. The three media are to be added to various dilutions as follows:

   (a) For bacteria—nutrient agar medium to plates with 10-^{4} to 10-^{7} dilutions.

   (b) For actinomycetes—glycerol yeast agar medium supplemented with aureomycin to plates with 10-^{3} to 10^{-6} dilutions.

   (c) For fungi—sabouraud agar medium supplemented with streptopenicillin to plates with 10-^{2} to 10-^{5} dilutions.

10. Upon solidification of the media, incubate all the plates in an inverted position at 25°C for 24 hours.

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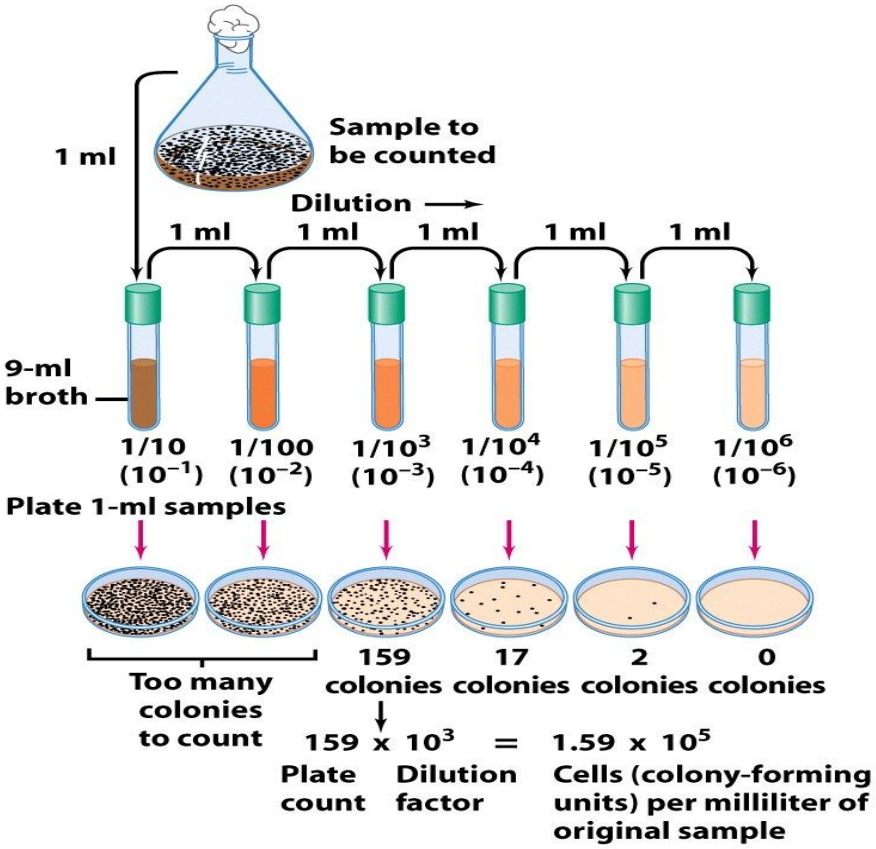

Fig 2. Diagram of the procedure of serial dilution

After isolation of micro organisms, the organisms were **stained** for visualization

Requirements:-

- 24-hour (or less) old cultures
- Gram staining reagents: Cristal violet, Gram iodine solution, 95 percent ethyl alcohol and Safranin
- Staining tray
- Distilled water
- Droppers

- Inoculating loop
- Glass slides
- Blotting paper/Absorbent paper
- Bunsen burner/spirit lamp
- Microscope.

Procedure:-

1. Make thin smears of S*taphylococcus* and *Escherichia* on separate Glass slides.

2. Let the smears air dry.

3. Heat fix the smears.

4. Hold the smears using slide rack or clothes pin.

5. Cover each smear with crystal violet for 30 seconds.

6. Wash each slide with distilled water for a few seconds, using washbottle.

7. Cover each smear with Gram's iodine solution for 60 seconds.

8. Wash off the iodine solution with 95 per cent ethyl alcohol. Add ethyl alcohol drop by drop, until no more color flows from the smear.
(The gram- positive bacteria are not affected while all gram-negative bacteria are completely decolorized).

9. Wash the slides with distilled water and drain.

10. Apply safranin to smears for 30 seconds (counter-staining).

11. Wash with distilled water and blot dry with absorbent paper.

12. Let the stained slides air dry.[13]

3. Results and Discussions:

Table1. Different test results

	N-HEXEN EXTRACT	ETHYL ACETATE EXTRACT	CHLOROFORM EXTRACT	ETHANOL	WATER
Test for steroid	+	-	-	+	-
Test for alkaloid	-	-	+	-	-
Test for glycosides	+	+	+	+	+
Test for protein	-	-	-	-	-
Test for flavonoids	+	-	-	+	+
Test for tannin	+	-	-	+	+
Test for carbohydrates	-	+	-	+	-

Observations:-

1. Plates which contain colonies in the range of 30 to 300 and make plate counts using a colony counter.

2. Since the dilution plates are replicates of each other, determine the average of the triplicate microbial counts.

OBSERVATION:-

1. Examine the slides microscopically using oil-immersion objective.

2. Identify the gram reaction of both the cultures and classify them.

3. Make sketches for morphology of the cultures.

4. Describe the morphology and arrangement of the cells.

Those bacteria that appear purple are referred to as Gram-positive; Those appearing pink are described Gram-negative. In S. a *ureus* the cocci appear dark purple or blue in colour, thus it is a gram-positive bacterium whereas in *E. coil* the rods appear pink and is thus a gram-negative bacterium. The organisms obtained as a result originally were staplococcus aureus.

In the present study I have done phytochemical evaluation and pharmacological evaluation to chake antimicrobial activity of n-hexen and ethanolic extract of Aerva Sanguinolenta .It would be better if I have done more evaluation of the extracts of Aerva Sanguinolenta. There will be scope of microscopic evaluation,physical evauation,biological evaluation of Aerva Sanguinolenta. Due to the lack of time I was unable to do more study.if I got more time, then I will work to find the active constituents responsible for antimicrobial activity of Aerva Sanguinolenta.

4. References:

[1]. Tariq O, Siddiqui, Javed Ahmed. Vitamin C content of Indian medicinal plants– A review literature. Indian Drugs 1985;23(2):72.

[2]. Modi A. Scope of Indigenous drugs in Modern Medicine. The Eastern Pharmacist 1984; 24(315):47-56.

[3]. Ayurveda: the Traditional Indian Medicine System and its Global Dissemination by D. P. Agrawal & Lalit Tiwar.

[4]. Farmworth NR. A Computerized Data Base For Medicinal Plants. The Eastern Pharmacist 1985; 28(326): 53-5.

[5]. Sampath K, Lakshmanan S. Indian System of Medicine. The Eastern Pharmacist 2001: 21-4.

[6]. Naik SR. Plant Derived Drugs. The Eastern Pharmacist 1986; 36:35-9.

[7]. Srimal, Shukla R. Recent Research in India on Indigenous Plants for the treatment of hypertension. Indian Drugs 1987; 24(9): 419-22.

[8]. Shah BN, Nayak BS, Seth AK, Jalalpure SS, Patel KN, Patel MN, Patel MA

and Mishra AD. Search of medicinal plants as a source of anti-inflammatory and anti-arthritic activity-a review. [Online]. 2006 Apr-Jun;[10 screens]. Available from: URL:http://www.vidyabhartitrust.org/pharmacy/material_anti-inflammatory.pdf

[9]. Pandey MM, Rastogi S, Rawat AKS. Indian Herbal Drug for General Healthcare: An Overview. [Online]. 2008;[13 screens]. Available from: URL: *Dept. of Pharmacognosy & Phytochemistry, K.L.E.S's College of Pharmacy, Belgaum* 64*Chapter IX Bibliography* http://www.ispub.com/journal/the_internet_journal_of_alternative_medicine/volume_6_number_1_2/article/html

[10]. Mukherjee PK. Ethnopharmacology in drug development. GMP for Botanicals. New Delhi: Business Horizons; 2003. p 99-112.

[11]. Patwardhan B. Ayurveda : the designer medicine: a review of ethnopharmacology and bioprospecting research. Ind Drugs 2000; 37(5): 213-27.

[12]. Khandlwal.K.R, Practical pharmacognocy,technique & experiment.9th edition,Pune;Nirali prakashan;2002.III.page 149-155

[13] Aneja.K.R,Experiments in Microbiology,Plant Pathology And Biotechnology,fourth edition,page 69-71

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***Corresponding Author:** Madhumita Dey, *GURUNANAK INSTITUTE OF PHARMACEUTICAL SCIENCE & TECHNOLOGY. 157/F, Nilgung Road, Panihati Kolkata- 700114. India.* Email: dey.madhumita05@gmail.com