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Antimicrobial Screening of Various Extracts of Rauvolfia Serpentina

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Abstract: Various extracts of Rauvolfia serpentina fruit evaluated for antibacterial and antifungal activity. The experimental methods were used cup plate method at a dose 1 mg/ml. Among the various extracts the water and chloroform extract showed good activity against bacterial strains E. coli and P. Klebsilla and remaining Pet. ether and Ethanol extract also showed good activity against P. aeruginosa and S. aureus. The various extracts of Rauvolfia serpentina were studied for antifungal activity among various extracts the Pet. Ether and Ehtanol extract showd good activity against A.flavus and A. niger and remaining extracts showed poor to moderate activity. All extracts of fruit subjected to Antimicrobial screening and the results are tabulated in Table (1) and (2).

Keywords: Rauvolfia serpentina, Antifungal, Antibacterial Screening.

I. INTRODUCTION

Rauvolfia serpentina or Sarpaghandha (Figure-1) plant is widely used medicinally both in the Modern Western Medical system and also in Ayurveda, unani and folk medicine. Sarpagandha is an important medicinal plant distributed in the foot-hills of Himalayan range, up to the elevation of 1300-1400 m. and almost all over the country. It is used in traditional medicine in India, China, Africa and many other countries. It helps to reduce blood pressure, depresses activity of central nervous system and acts as a hypnotic. In India and Nepal, it is a common treatment for hypertension and insomnia. Hindus used this plant for centuries as a febrifuge and as an antidote to the bites of poisonous reptiles like snakes[1-6]. Now it's medicinal value has been accepted by the allopathic system.

II. MORPHOLOGY OF PLANT

Morphology Description (Habit) It is an evergreen, perennial and erect undershrubs grows up to a height of 60 cm (rarely more than it). Roots are tuberous with pale brown cork. Leaves are in whorls of three, elliptic to lanceolate bright green above, pale green below, tip acute or acuminate, base tapering end slender, petioles long. Flowers are many irregular flowered. Peduncles long but pedicels stout. Flowers white, often has violet coloured tinge.Corolla is longer than calyx, tube slender, swollen a little above the middle, lobes 3, and elliptic-oblong. Disc is cup shaped. Drupes are slightly connate, obliquely ovoid and purplish black in colour[7-8].

III.CHEMICAL CONSTITUENT

The major alkaloid present in root, stem, leaves and fruits of the plant is Reserpine varies from 1.7 to 3.0 %. The root barks has more than 90% of the total alkaloids in roots. The minor alkaloids present in the plant are Ajmalicine, ajmaline, isoajmaline, ajmalinine, chandrine, rauwolfinine, renoxidine, rescin-namine, reserpiline, reserpin, reserpinine, sarpagine, serpentine, serpentinine, yohimbine Tetraphyllicine and 3-epi-a-yohimbine. The root contains ophioxylin, resin, starch and wax[9].

Actions:

- Reduces cardiac output.
- Relaxes capacitance vessels and reduces total peripheral resistance.
- Cause sedative effect

IV.MEDICINAL PROPERTIES

1. Sarpagandha acts on mild to moderate hypertension.
2. In case of insomnia sarpagandha is used with pesution.
3. It is used as a tranquilizer in psychiatric disorder.
4. Sarpagandha has extensive use as an antidote for snakebites.
5. The root is believed to stimulate uterine contraction, recommended for use in child birth.
6. It is used in treatment of intestinal disorders, particularly diarrhea and dysentery, It is used in treatment of intestinal disorders, particularly diarrhea and dysentery, cholera, colic and fever.It is also an antihelmintic.
7. The juice of the leaves has been used as remedy for opacity of the comea[10]

Fig. 1 Rauvolfia serpentina

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Collection and drying of fruits:
The fresh fruits of Rauvolfia serpentina were collected during December 2009, in and around Gulbarga University. The collected fruits were immediately sprayed with alcohol (95%) to cease the enzymatic (activity) degradation. They were chopped, chopping fresh materials at once after collection fastens drying and advantageous reduction in bulk and drying speed. The fruits were kept for 15 - 20 days and are then grinded into fine powder.

Extraction of the fruits of Rauvolfia serpentina:
The powdered material (200 g) was extracted with petroleum ether, chloroform ethanol and water in Soxhlet extractor exhaustively for 48 hours each. The extracts were concentrated at reduced temperature and controlled temperature (40-50º). The petroleum.ether extracts yields yellow color gummy product (13.63 g), chloroform extract yielded dark brown colored sticky substance (1.53 g), ethanol extract yielded dark brown colored sticky substance (11.39 g) and water extract yields brown color gummy substance (20.78 g). The crude extracts were kept in desiccators and stored in refrigerator. These extracts were taken for antibacterial and antifungal activities.

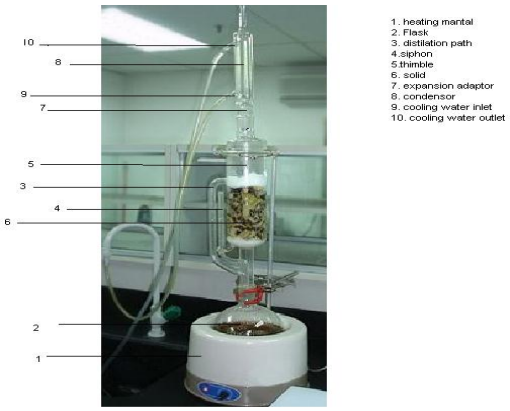

Fig. 2 Soxhlet Apparatus

V. ANTI-BACTERIAL ACTIVITY

Plant products are gaining prominence as bactericides and fungicides in view of their systemic activity and low photo-toxicity. A large number of plants are known for their antibacterial and antifungal activity, hence, an attempt was made to study the antibacterial and pharmacological properties of different extracts of Rauvolfia serpentina.

Materials and methods:
Antibacterial activity of different extracts of Rauvolfia serpentina was assessed against E-coli, Klebsiella, Pseudomonas and S. Aureus. The following materials are used Nutrient agar medium, Sterilized Petri dishes, Pipettes of 0.1 – 0.2ml, Cultures, nutrient broth and Sterilized test tubes containing solutions of extracts of known concentration.

Preparation of Media:
Nutrient agar medium was prepared by dissolving bacteriological peptone (5g), Beef extract (3g), sodium chloride (3g) and agar (20g) in distill water to produce one liter of medium. Then, it was sterilized for 30 minutes at 15 lbs pressure. The pH of the solution was adjusted to 66-70 by using 40% NaOH and HCl.

Preparation of Sub-cultures:
The organisms used in the present study for testing the antibacterial activity of the extracts were obtained from the laboratory stock. On the day of testing, the organisms were sub-cultured into sterile nutrient broth. After incubating the same for 3 hour, the growth thus obtained was used as inoculums for the test.

Sterilization of media:
The media used in the present study, nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving at 15 lbs pressure for 20 minutes. The cork borer, petridishes, test tubes and pipettes were sterilized by employing hot air oven at 160ºC for 1 hour.

Preparation of test solutions:
Solutions of all the extracts were prepared in distilled DMF and tested at the concentration 1mg/ml.

Method of testing – Cup plate method:
With the help of a sterile borer, five cups of each 8mm diameter were punched and scooped out of the test agar (the cups were numbered for the particular concentration of extracts and standards). Using sterile pipettes 0.1ml, of the prepared standard and the sample solution were feel into bored cups. The dishes were left standing for 1-4 hour at room temperature as a period of pre-incubation of the different solutions. These were then incubated for 24 hours at 37ºC. The zone of inhibition developed, if any, was then accurately measured and recorded.

TABLE I (Antibacterial activity)

Extracts	Diameter of zone in mm			
	E.coli.	P. Klebsiella	P. aeruginosa	S. aureus
Pet.ether extract	15	13	20	10
Chloroform extract	14	18	19	8
Ethanol extract	16	14	12	16
Water extract	20	12	13	14
Standard Gentamycin	21	20	22	17

*Zone of inhibition excluding well size 6mm

VI. ANTI-FUNGAL ACTIVITY

The antifungal activity of plant extracts in comparison with that of standard antifungal drugs flucanozole by cup-plate method the fungi selected for this were of A. flavus and A. niger.

Cup-plate diffusion Method:
The antifungal activity of the test compounds was assessed against A. terrus, A-niger, A-cleavatus and A. flamp by

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plate diffusion method.

The following materials are required.Nutrient agar medium, Sterilized Petri-dishes, Pipettes of 0.1ml and 0.2ml, Cultures and nutrient broth and Sterilized test tubes containing solution of test compounds at known concentration.

Preparation of media:

Nutrient agar medium was prepared by dissolving bacteriological peptone (5g), Beef extract (3g), Sodium chloride (3g) and agar (20g) in distill water to produce one litre of medium. Then, it was sterilized for 30 minutes at 15 lbs pressure. The pH of the solution was adjusted to 6.6-7.0 by using 40% NaOH and HCl.

Sterilization of media:

The media used in the present study, nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving at 15 lbs pressure for 20 minutes. The cork borer, Petri dishes, test tubes and pipettes were sterilized by employing hot air oven at 160ºC for 1 hour.

Preparation of solutions of Extracts:

Solutions of all the extracts were prepared in distilled DMF and tested at the concentration 1mg/ml.

Cup-plate method:

This method reveals that diffusion of an antifungal from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing the solution of antifungal. A previously liquefied nutrient medium was inoculated appropriate to the assay with the requisite quantity of suspension of the microorganism and the suspension to media at the temperature between 40-50ºC and immediately poured the inoculated medium into Petri dishes to give a depth of 3-4mm. Ensure that the layers of the medium were uniform in thickness by placing the dishes on the levelled surface. The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before dishes were used and that the surface of agar layer was dry at the time of use. With the help of sterile borer, five cups of each 6mm diameter were punched and scooped out of the set agar (the cups were numbered for the particular concentration of extracts and standard).

TABLE II (Antifungal activity)

Organism	**Zone of inhibition in mm (48 hours)**				
	Pet ether extract	**Chloroform extract**	**Ethanol extract**	**Water extract**	**Standard 1mg/ml**
A.niger	12	13	**14**	12	15
A. flavus	**22**	18	11	10	22

*Zone of inhibition excluding well size 6mm

Using separate sterile pipettes 0.1ml of the prepared standard and sample solution were feel into bored cups. The dishes were left standing for 1-4 hours at room temperature as a period of pre-incubation diffusion to minimize the affects of variation in time between the applications of different solution. These were then incubated for 24 hours at room temperature. The zone of inhibition developed if any was then accurately measured.

VII. RESULTS AND DISCUSSION

The various extracts of Rauvolfia serpentina screened for antibacterial and antifungal activity. Among the various extracts the water and chloroform extract showed good activity against bacterial strains E.coli and P.klebsilla and remaining Pet. ether and Ethanol extract also showed good activity against P. aeruginosa and S. Aureus. For antifungal activity among the various extracts the Pet. Ether and Ehtanol extract showd good activity against A.flavus and A. niger and remaining extracts showed poor to moderate activity.

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