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Antibactreial activity of fruits of *Randia Dumotarum Lamk*

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ABSTRACT

The aim of present work was to check the anti-bacterial activity of extract of a crude product Randia dumetorum fruit by using disc diffusion method by quantitative manner. Till today all anti-bacterial activity of Randia dumetorum was carried out on qualitative manner but we give reading according to quantitative manner. The maceration of powder fruit of Randia dumetorum can be done in methanol & water (water extract).Methanol extract is fractionated by using various polar solvent such as petroleum eater, chloroform ethyl acetate and methanol,that concentrated extract was collected and screened for anti-bacterial activity.

Keywords:-Randia dumetorum, mucilage, ampicilline, Disc diffusion.

INTRODUCTION

Medicinalplantare well known as natural sourceof remedies for treatment of various diseasessinusantiquity. According to report by WHO nearby 20,000 plant species are currently used for medicinal purpose. Over use of antibiotics has resulted in increasing resistant for bacteriaagainst theses drugand also causes Nemours side effect in human. Since some herbal are antibacterial qualities .They could be used as harmless substitutes for antibiotics in treatment of various disease .The use of medicinal herb in world contribute significantly to primary health care[1].

Randia dumetorum Lamk. (Rubiaceae) known as Madana (Sans), Mainphal (Hindi), Emetic nut (Eng)[2].A large deciduous thorny shrub grows up to 5 meters of height. Leaves simple, obviate, wrinkled, shiny and pubescent.Flowers white, fragrant, solitary, seen on at the end of short branches.Fruits globes, smooth berries with longitudinal ribs; yellow when ripe. Seeds many, compressed, and embedded in the dark fruit pulp. Its fruits are considered to be tonic, demulcent, diuretic and restorative, the drug is claimed as a medical cure for piles, anti-dysenteric agent, asthma, jaundice, diarrhea, emetic and gonorrhea[3].It shows also a following activity such as anti-tumor,anti-inflammatory,anti-fertility,insecticidal,anthalmetics,immenomodulatory,analgesic[4,5,6,7,8,10].

MATERIALS AND METHODS

The fruits of Randia dumetorumwere purchased from local market from Pune and authenticatedFrom Botanical Survey of India, Pune.

All other reagents and chemicals required for the study were of AR grade.

Plant material and extract preparation:-
Dried fruits of R. dumetorumwere purchased from local market,Pune, India. The fruits were identified and authenticated fromBotanical Survey of India, Pune, India and assigned voucherspecimen no BSI/WC/Tech./2006/622). Maceration of air-driedpowdered fruits of R. dumetorumafforded water extract(30% w/w) methanolextract (17w/w). Methanol extract so obtained was thenfractionedbymaceration into different polarity solvents like petroleum ether, chloroform,ethyl acetate and methanol.All respective fractions wereconcentrated under vacuum.

Phytochemical screening:-
Phytochemical screening was done for methanol extract and itsfraction[9].Methanol extract gave positive testsfor alkaloids, Phenolic, steroids, terpenoids, saponins, flavonoidsand carbohydrates. Petroleum ether and chloroform fraction gavepositive tests for terpenoids and steroids[8]. Ethyl acetate fractiongave positive test for flavonoids, terpenoids, steroids and tanninswhereas methanol fraction gave positive tests for saponins,alkaloids, phenolic, flavonoids and carbohydrates on phytochemicalScreening.

Anti-bacterial activity:-
The compounds prepared during the present investigation were screened for their antibacterial activity. The antibacterial tests were conducted on four common microorganisms such as *Bacillus subtilis*,*staphylococcusaureus*,and*Escherichiacoli*,*salmonellatyphi*, which are the representative types of gram positive and gram-negative organisms respectively. The antibacterial activity of the compounds was assessed by disc diffusion method.

Materials used:Sterilized Petri dishes, Sterilized 6mm cork borer, Sterilized inoculation loop , Sterilized test tubes, graduated pipettes and watch glasses, Sterile tubercular syringes, Sterilized fine pointed forceps, Sterile tubercular syringes.

Preparation of Nutrient broth:

Composition:

Peptone (Bacteriological)	: 20 g.
Beef extract (Bacteriological)	: 5 g.
Sodium Chloride	: 5 g.
Distilled water up to	: 1000 ml.

Nutrient broth is prepared by dissolving all these and steam for about 2 hour adjust the reaction mixture pH to about 7.2 and autoclave at 15 lbs pressure for 20 minutes. One day prior to the testing, the organisms obtained from the laboratory stock were subculture into sterile nutrient broth and incubated at 37^{o}C for 18-24 hours. The culture growth thus obtained was used as inoculums for the antibacterial testing.

Preparation of nutrient agar media: Preparation of nutrient agar media:
The nutrient agar media was prepared by using the following ingredients.

Peptone (Bacteriological)	: 20 g.
Beef extract (Bacteriological)	: 5 g.
Sodium chloride (Bacteriological)	: 5 g.
Agar (Bacteriological)	: 20 g.
Distilled water up to	: 1000 ml.

Weighed quantities of peptone, beef extract were dissolved in distilled water by gentle warming, and then the specified amount of agar was dissolved by heating on boiling water bath. Then the pH of the above solution is adjusted by adding sodium chloride and the volume of final solution is made up to 1000 ml with distilled water. Then the above prepared nutrient agar media is sterilized by autoclave at 121oC for 20 minutes at 15 lbs./in2 pressure.

Preparation of test solution:We prepare solution by dissolving a crude drug extract in a suitable solvent such as methanol extract 100ug are dissolved in methanol and chloroform extract 100ug in chloroform (100ml)and carried

out anti-bacterial activity of following drug.These sample solutions were made in suitably labeled sterilized test tubes.

Preparation of standard solution:
The standard drug used in this testing is Ampicillin. It is water-soluble; the concentration of this drug is adjusted so as to contain 100ug/ml.

Method of testing:
The above prepared nutrient agar media is cooled to 45^{o}C with gentle shaking to bring about uniform cooling. To this 0.5 – 0.6 ml of 18-24 hours old culture was injected aseptically and mixed well by gentle shaking. This was poured onto the Petri dishes and was allowed to set for 1 hour.

Thereafter the cups were made by punching into the set agar with a sterile cork borer and scooping out the punched part of the agar. The diameter of each cup was 6mm. To these cups 100 µl of the test compound was put, which was prepared in methanol extract. After adding the drugextract solution, it was allowed to diffuse for about 45 minutes, at room temperature. Then the plates were incubated at 37^{o}C for 24 hours in an incubator.

The extent of diameter of inhibition after 24 hour was measured as the zone of inhibition in millimeters.

Serial number	SAMPLE	Escherichia coli (gram - ve)	Bacillus subtilis (gram + ve)	Salmonella typhi (gram – ve)	Staphylococcus aureus (gram + ve)
01	WATER EXTRACT(A)	02	08	08	02
02	METHANOL EXTRACT(B)	09	02	13	09
03	PETRIEATHER FRACTION(C)	12	15	12	15
04	CHLOROFORM FRACTION(D)	12	11	15	15
05	ETYL ACETATE FRACTION(E)	03	02	08	12
06	METHANOL FRACTION(F)	03	11	10	08
07	AMPLICILLINE(G)	15	16	18	18

**Average of triplicate ± Standard deviation*
Note: '-'denotes no activity, 02-07 mm poor activity, 08-13 mm moderate activity,13-18 above good.

RESULT AND DISCUSSION

Antibacterial activity:
All the prepared compounds were screened for antibacterial activity studies at a concentration by using DMSO as a control against Escherichia coli, Bacillus subtilis,Staphylococcus aureus andSalmonella typhi by disc diffusion method on nutrient agar media, Ampicillin 100ug/ml used as standard against Gram positive and Gram negative bacteria. The data in table indicates that some of the prepared agent agents are not active against bacteria such as water extract. But the other extract shows moderateactivity(methanol extract, ethyl acetate fraction and methanol fraction) and other shows very good anti-bacterial activity (Pet ether and chloroform fraction).

The highest antibacterial activity found with chloroform and Pet ether fraction against all investigated Phytopathogenic bacteria.

The compound constituents responsible for antibacterial activity were not investigated .how ever preliminary phytochemical screening of petroleum ether and chloroform fraction give positive test for terpenoids and steroids. The role of terpenoids and steroids as antibacterialagent[14].

Previous report shows that oleanoicacid was isolated for seed fruits of *Randia dumetorum* .Recently it shows that oleanoic acid is well known antibacterial agent[13].
The activity of chloroform fraction may attribute to presence of steroids andolanoic acid which is reported in Randia dumetorum.

The present study reveals that Randia dumetorum has anti-bacterial activity and chloroform fraction is bioactive fraction of plant.

This work for development of herbal medicine in progress worldwide ,the present work help in isolation of new product.

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