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Research Paper

SCREENING OF TEN INDIAN MEDICINAL PLANTS FOR THEIR ANTIBACTERIAL ACTIVITY AGAINST *SHIGELLA SPECIES* AND *ESCHERICHIA COLI*

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Abstract

Ethanol and Aqueous extracts of ten Indian medicinal plants were tested for their antibacterial properties against *Shigella sonei, S. boydi, S. flexeneri, S. dysenteriae* and *Escherechia coli* with disc diffusion, well diffusion, and minimum inhibitory concentration methods. The results showed that the aqueous extract of the bulb of *Alium sativum* was effective against *S. dysenteriae, S. boydi,* and *E. coli*. The Ethanol extract of the rind of *Garcinia mangostana* showed activity against *S. sonei*. The results were compared with results obtained using standard antibiotic disc (Chloramphenicol 30 ug/disc and Ciprofloxacin 5 ug/disc). Spectral analysis of the crude aqueous extract of *A. sativum* and the crude ethanol extract of *G. mangostana* were carried out.

Key words: Antibacterial activity, medicinal plant, plant extract, Zone of inhibition, Disc diffusion .

Introduction

India is rich in medicinal plants. Over 25% of our common medicines contain at least some compounds obtained from plants. The World Health Organization reported that 80% of the worlds population relies chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents. Due to the indiscriminate use of antimicrobial drugs microorganisms have developed resistance to many antibiotics. This has created immense clinical problems in the treatment of infectious diseases (Davis 1994).

In India herbal medicines have been the basis of treatment and cure for various diseases / physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Although antibacterial activities of indigenous plants have been reported from many regions, they have not been systematically conducted except in a few cases thereby leading to confusion in drawing meaningful conclusions. (Padmaja et al. 1993; Ndamba et al. 1994; Vijaya et al. 1995).

Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes that may be for healing wounds, treating inflammations due to infection, skin lesions, leprosy, diarrhea, scabies, venereal diseases, snake bites, ulcers etc. What is interesting however is that quite often a particular plant may be used for different diseases. For example the decoction of *Sebastiana chamaela* is considered a tonic for diarrhea in India and China (Caius 1937) while the astringent effect of the juice is applied externally to cure leprosy (Perumal swamy et al. 1998).

Materials and Methods

Preparation of Plant extract

Ethanol Extract

The method of Saxena et al. (1993) was adopted for the preparation of plant extracts. The parts of the selected plants were shade dried till they became crisp. They were powdered in pestle and mortar. 1 gram of the

powder was soaked with 10 ml of ethanol for 24 hrs. The filtrate was dried at 50°C. 100 mg of the extract was scraped and dissolved with 0.02 ml Tween 80, 0.02 ml ethanol and 0.98 of distilled water (i.e.). 100 mg in 1 ml. Tween 80 was added to dissolve the organic compounds. The extracts were filter sterilized. A mixture of 0.02 ml of Tween 80, 0.98 ml distilled water and 0.02 ml of ethanol was prepared and used as control. The details such as common name, botanical name, family, different parts and extracts used are given in Table 1.

Aqueous extract

The parts of the selected plant were shade dried till they became crisp. They were powdered in pestle and mortar. 1 gram of the powder was soaked in 10 ml of sterile distilled water for 24hrs. The filtrate was dried at 50°C. 100 mg of the extract was scraped and mixed with 1 ml of sterile distilled water. The extract was filter sterilized and used. Sterile distilled water was used as control.

Well Diffusion method

The media and chemicals used in the study were products of Hi Media Laboratories Pvt, Ltd., Mumbai. Clean Borosil glass wares were used. The cleaned glass wares, swabs and well cutter were sterilized in and autoclave at 121°C for 15 min. Bacterial strains *Shigella sonei, S. boydi , S. flexeneri, S. dysenteriae* and *Escherechia coli w*ere obtained from the Department of Microbiology, Christian Medical College and Hospital, Vellore, Tamilnadu, India. The bacterial cultures used in the study were regularly sub cultured and maintained at 4°C. The required quantity of the medium was prepared according to the manufacturer's instruction. The medium was sterilized and poured into a sterile Petri plate and allowed to solidify at room temp. The agar well diffusion method (Perez et al. 1990) was followed. Using a sterile cotton swab lawn cultures of the test organisms were made on the Muller Hinton agar plates. (*S. sonei, S. boydi, S. flexeneri, S. dysenteriae* and *E. coli)*. Two wells of 8 mm diameter were punched into the agar on each plate using a sterile well cutter. Into one well in each plate 30 µl of the plant extract was added. The solution was allowed to diffuse for 2 hrs. The plates were incubated at 37ºC for 24 to 48 hrs. The antibacterial activity was evaluated by measuring the zone of inhibition around the well.

1. Control for ethanol extract: 0.02 ml Tween 80 + 0.98 ml of distilled water + 0.2 ml of ethanol.
2. Control for aqueous extract: Sterile distilled water.

Preparation of Disc

What man No: 1 filter paper was punched into discs using a paper punch. The discs were sterilized. 10 µl of the prepared plant extract was added to the disc and dried under sterile condition at room temperature. The prepared discs were used in the study.

Comparative Study

The antibiotic sensitivity test was carried out by the method of Bauer et al (1966). The antibiotic discs were obtained from Himedia Laboratories Limited, India. Two antibiotic discs were used. They are Chloramphenicol (30 mcg/disc) and Ciprofloxacin (5 mcg/disc). Bacteria that were tested for their sensitivity against antibiotics were cultured in sterilized Muller Hinton agar. Approximately 15 ml of the sterile medium was poured into sterilized Petri plates and allowed to solidify. The test organism was transferred from stock into glass tube containing 5 ml of sterile nutrient broth with the help of a sterile wire loop. The inoculated broth was incubated at 37°C for 24 hrs. Broth cultured bacteria were swabbed on Muller Hinton agar plates under sterile condition. The plates were kept at room temp for 10 min for drying under strict aseptic condition.

The antimicrobial susceptibility discs were carefully dropped on to the surface of the Muller Hinton agar plates using sterile forceps. The extract loaded discs were applied to the lawn cultures of test organisms. The plates were incubated at 37°C for 48 hrs. The agar plates were examined for circular clear area in the bacterial lawn around the disc.

Minimum Inhibitory Concentration

The procedure described by Pelczar et al. (1986) was followed. To determine the minimum inhibitory concentration of the plant extracts which gave positive results, the extracts were added in increasing amounts in

nutrient broth. Equal amount of Bacterial suspension of the test organism was added to each of the tubes. The mixture was allowed to incubate overnight and the turbidity in each was visualized. The highest dilution of the plant extract in which there was no growth of the organism on the nutrient broth was determined.

Thin Layer Chromatography

20 grams of Silica gel and 40 ml of distilled water were mixed to form slurry. The slurry was poured on to a clean grease free glass plate and spread evenly. This was dried in a hot air oven at 50 ºC. The plate was taken out. A pencil line was drawn at a distance of 2 cm from the bottom of the glass plate. Three drops of the extract that gave positive result were applied on to the plate as small spots using a capillary tube.

A glass tank was taken and filled with 100ml of solvent (n-butanol, acetic acid and water in ratio 40:10:50). The plate was suspended in the chromatography tank in such a way that the spots did not touch the solvent directly. The set up was left until the solvent front moved to about 2 cm below the top edge of the plate. After this it was taken out and dried. 5 % of ethanolic ferric chloride was sprayed for the positive extracts. The colour developed was noted.

Ultra Violet Spectral measurement

The ultraviolet spectra of the crude compound obtained from *Allium sativum* and *Garcinia mangostana* were recorded at 200 - 400 nm on a Shimadzu (UV 1601) instrument using double distilled water as a solvent for *Allium sativum* and ethanol as solvent for *Garcinia mangostana*.

Results

The aqueous extract of the bulb of *Allium sativum* 600 mg/6ml concentration tested against *Shigella dysenteriae, S boydi, S flexeneri, S sonnei and Escherichia coli* by disc diffusion method for its antibacterial activity showed that it had maximum effect against *S dysenteriae*. The disc diffusion method showed zone of inhibition of 15 mm diameter (24 mm for standard antibiotic disc Choloramphinicol 30 mcg/disc). *S boydei* and *Escherichia coli* were found to be sensitive. Zone of inhibition was 14 mm for both (26 mm for standard disc Ciprofloxacin 5 mcg / disc for *E.coli*). No antibacterial activity was seen against *S sonei* and *S flexeneri.* Well diffusion method showed 14 mm diameter zone of inhibition for *S boydi* and S *dysenteriae* and 12 mm for *Escherichia Coli*. Results are tabulated in Table 2 and Table 3

The ethanol extract of the rind of *Garcinia mangostana* tested against *Shigella sonnei, S boydi, S flexeneri, S dysenteriae* and *Escherechia coli* by disc diffusion method for its antibacterial activity showed inhibitory effect against *S sonnei.* The zone of inhibition was found to be 11 mm diameter (24 mm diameter for standard disc Chloramphenicol 30 mcg/disc). No inhibitory activity was found against the other four organisms. Well diffusion method showed 9mm diameter zone of inhibition for *S sonnei.* No inhibitory activity was found against the others. Table 4 and Table 5

The MIC value of *Allium sativum* and *Garcinia mangostana* were determined. The concentration of crude aqueous extract of *Allium sativum* taken for the experiment was 600mg/6ml. At a volume of 40 microliters of crude extract *Shigella dysenteriae, S boydii and Escherichia coli* show weak growth whereas at a volume of 50 microliters all three of them do not show any growth. The control was bacterial culture without extract.

The concentration of crude ethanol extract of *Garcinia mangostana* taken for the experiment was 65mg/650 microliters. At volumes of 50 and 60 microliters of crude extract *S sonnei* shows weak growth whereas at a volume of 70 microliters it does not show any growth. The control was bacterial culture with out extract. Table 7 and Table 8

The compound identified in the case of Garcinia *mangostana* was a Flavonoid. In the case of *Allium sativum* as no colour developed absence of Flavonoid was concluded. Table 6

The crude aqueous extract of *Allium sativum* was found to have an absorption maximum at 293.2 nm and corresponding absorbance was noted at 1.376.

The crude ethanol extract of *Garcinia mangostana* was found to have an absorption maximum at 318nm and corresponding absorbance was noted at 1.021.

Discussion

Due to indiscriminate use of antimicrobial drugs microorganisms develop resistance to many antibiotics. In addition to this many of them are known to have side effects. Therefore there is a need to screen local medicinal plants for possible antibacterial properties. With this aim ten Indian medicinal plants with known medicinal

Table 1: Plant Parts tested and extract used

S.No	Common Name	Botanical Name	Family	Part tested	Extract
1	Garlic	*Allium sativum*	Liliaceae	Bulb	Aqueous
2	Shoe Flower	*Hibiscus rosasinensis*	Malvaceae	Flower	Ethanol
3	Mangosteen	*Garcinia mangostana*	Guttiferae	Rind	Ethanol
4	Noni	*Morinda citrofolia*	Rubiaceae	Leaf	Ethanol
5	Indian Blackberry (Jamun)	*Syzygium cumini*	Myrytaceae	Bark	Aqueous
6.	Pomegranate	*Punica granatum*	Puniceceae	Fruit	Ethanol
7	Onion (small variety)	*Alliumcepa*	Liliaceae	Bulb	Aqueous
8	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome	Aqueous
9	Papaya	*Carica papaya*	Carcicaceae	Seed	Ethanol
10	Neem	*Azadiraehta indica*	Meliaceae	Bark	Ethanol

Table 2: Antibacterial activity of *Allium sativum* by Disc diffusion method

S.No.	Name of the organisms	Standard Antibiotic disc	Diameter of Zone of Inhibition	Diameter of Zone of inhibition for extract loaded disc
1	*Shigella dysenteriae*	Chloramphenicol	24	15
2	*Shigella boydii*	Chloramphenicol	24	14
3	*Escherichia coli*	Ciproflaxacin	26	14
4	*Shigella sonnei*	Chloramphenicol	24	No activity
5	*Shigella flexeneri*	Chloramphenicol	24	No activity

Table 3: Antibacterial activity of *Allium sativum* by well diffusion method

S.No	Name of the organisms	Diameter of zone of inhibition
1	*Shigella dysenteriae*	14
2	*Shigella boydii*	14
3	*Escherichia coli*	12
4	*Shigella sonnei*	No activity
5	*Shigella flexeneri*	No activity

properties were tested for their antibacterial activity against *Shigella sonnei, S boydi , S flexeneri , S dysenteriae* and *Escherechia coli*. Monira et al. (1996) tested the effects of an aqueous extract of *Allium sativum* and its active constituent Allicin against isolates of S*higella dysenteriae* type 1, *S flexeneri, Escherchia coli* and *Vibrio cholerae*.

Table 4 : Antibacterial activity of *Garcinia mangostana* by Disc diffusion method

S.No	Name of the organism	Standard antibiotic disc	Diameter of Zone of Inhibition	Diameter of Zone of inhibition for extract loaded disc
1	*Shigella dysenteriae*	Chloramphenicol	24	No activity
2	*Shigella boydii*	Chloramphenicol	24	No activity
3	*Escherichia coli*	Ciproflaxacin	26	No activity
4	*Shigella sonnei*	Chloramphenicol	24	11
5	*Shigella flexeneri*	Chloramphenicol	24	No activity

Table 5 : Antibacterial activity of *Garcinia mangostana* by well diffusion method

S.No	Name of the organism	Diameter of zone of inhibition
1	*Shigella dysenteriae*	No activity
2	*Shigella boydii*	No activity
3	*Escherichia coli*	No activity
4	*Shigella sonnei*	No activity
5	*Shigella flexeneri*	No activity

Table 6 : Thin Layer chromatography

S.No.	Extract	Colour developed	Compound
1	*Garcinia mangostana*	Bluish grey	Flavonoid
2	Allium sativum	No colour	Absence of Flavonoid

Table 7: Determination of the minimum inhibitory concentration of *Allium sativum (concentration 600 mg / 6* ***ml).***

S. No.	Organism	Control	Volume of the crude aqueous extract in microlitre			
			20	30	40	50
1	*Shigella dysenteriae*	+	+	+	±	-
2	*Shigella boydii*	+	+	+	±	-
3	*Escherichia coli*	+	+	+	±	-

+ ----- Indicates the growth
- ----- Absence of growth
± ----- Weak growth
Control: **Bacterial culture without extract.**

Table 8: Determination of the minimum inhibitory concentration of *Garcinia mangostana (concentration 65* ***mg / 650 ml).***

S.No.	Organism	Control	Volume of the crude ethanol extract in microlitre					
			20	30	40	50	60	70
1	*Shigella sonnei*	+	+	+	+	±	±	-

+ ----- Indicates the growth
- ----- Absence of growth
± ----- Weak growth
Control: Bacterial culture without extract.

The extract and Allicin exhibited activity against all of the bacteria tested. Allicin was reported to be more potent. The results of the present study indicated that *S dysenteriae, S boydii and E coli* were sensitive to *Allium sativum*. The Zone of Inhibition was 14mm, and 12mm for S *boydi* and *Escherchia coli* respectively by well diffusion method. The disc diffusion method showed zone of inhibition 15 mm, 14mm and 14mm at 1mg/disc for *Shigella dysenteriae, Shigella boydi* and *E. coli* respectively. (Zone of inhibition for *Shigella* species at 30 mcg/disc and 26mm for *E coli* at 5mg/disc for standard disc).

The minimum inhibitory concentration of the extract required to control the bacterial load was found to be 5mg for all the organisms which tested positive. This result differs slightly from the results reported by Monira et al. (1996) in that the aqueous extract of *Allium sativum* in the present study did not show any effect against *Shigella flexeneri*. However it showed positive results against *S. boydii*. This differs from the results reported by Monira et al. (1996). Gopalakrishnan et al. (1977) studied the antifungal activity against several *Xanthomonas* sps isolated from the fruit hull of *Garcinia mangostana* and some derivatives of mangosteen against three pathogenic fungi *Fusarium oxysporum* var *infectum*, *Alternaria tenuis* and *Dreschiera oxyzae*. They reported that natural xanthones showed good inhibitory activity against these three fungi.

The present study showed that the ethanol extract of the rind of mangosteen showed antibacterial activity against S*higella sonnei*. Zone of inhibition was 9mm for well diffusion and 11 mm for disc diffusion at 1mg/disc (standard disc at 30mcg/disc for S*higella species* showed 24 mm zone of inhibition). The minimum inhibitory concentration of the extract required to control the bacterial load was found to be 7mg for *S sonnei*. This finding differs from the report of Goplakrishnan et al. (1977) in that the present study showed that *G. mangostana* has antibacterial activity also. Toxicity tests of the active compounds of *Alium sativum* and G*arcinia mangostana* using an animal model have to be carried out before we can consider using them as natural antibiotics.

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