A STUDY OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF VARIOUS PLANT LEAVES AGAINST SELECTED MICROBIAL SPECIES

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Abstract

To evaluate the antimicrobial activities of extract of leaves were examined against four common bacterial isolates. The ethanolic extracts of various leaves such as *Moringa oleifera* (Murungai) , *Musa paradisiaca* (Banana), *Azardiratica indica* (Neem), *Cynodon dactylon*(Grass), *Alternanthera sessilis* (Ponnangkani), *Anisochilus carnosus* (Karpooravalli), investigated individually for antimicrobial activity by disc diffusion method .These were investigated against selected species of *Escherichia coli, Bacillus subtilis, Vibrio cholerae, Klebsiella pneumoniae* to find the inhibitory activities of the microbes. The ethanolic extract of *Azardiratica indica* showed considerably high activity against *Escherichia coli* than other extracts. These results were compared with standard antibiotic Penicillin. But the extract showed higher activity than the given standard antibiotic.

Keywords: *Moringa oleifera, Musa paradisiaca, Azardiratica indica, Cynodon dactylon, Alternanthera sessilis Anisochilus carnosus.*

Introduction

Concern has been expressed about the rising prevalence of pathogenic microorganisms, which are resistant to the newer or modern antibiotics that have been produced in the last three decades (*Cohen,* 1992; Nascimento et al., 2000). Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized (*Shariff,*001).Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (*Nascimento et al.,* 2000; *Rios and Recio*, 2005).

For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents (*Iwe et al., 1999*).

Materials and methods

Plant extracts preparation

The plant materials used were collected and fresh leaves were isolated and dried in shade. The dried leaves were ground to powder and suspended in petroleum ether and kept in refrigerator overnight for removing all the fatty substances. After overnight incubation, the supernatant was discarded and the residue was dried at room temperature. The residue was further divided in to two parts and each part was suspended in ethanol and ethyl acetate respectively in sterile 25 ml conical flasks and kept at 4^{o}C overnight.

After overnight incubation, the supernatant was filtered through Whatman No.1 filter paper and the filtrate was dried to evaporate the organic solvent at room temperature. The sedimented extract was weighed and dissolved in 5% dimethyl sulfoxide (DMSO).

Microorganisms

Escherichia coli, Bacillus subtilis, Vibrio cholerae, Klebsiella pneumoniae was obtained from the Microbiology Laboratory of our college. Standard strains of microbes were used which was incubated at 25ºC for 24

- 48 h. Each of the 24 h old pure culture was suspended in a Roux bottle containing 5 ml of physiological saline. Each suspension of microorganisms was standardized to 25% transmittance at 560 nm using an ultraviolet (UV) - visible spectrophotometer.

Antimicrobial screening

The modified (*Collins et al*. 1995) agar-well diffusion method was employed to determine the antimicrobial activities for both ethanolic and aqueous extracts. One millilitre (1 ml) of ethanolic extracts of the given leaves was used against the test microorganisms.

Approximately 10 ml of sterile Muller-Hinton Agar (MHA) was poured into sterile culture plates and allowed to set. About 10 ml of the antibiotic medium No. 2 seeded with 0.5 ml of a 24 h old culture of bacteria isolates was layered onto the MHA and allowed to set.

The seed medium was then allowed to dry at room temperature for about 30 min. With the aid of a sterile cork borer, wells of about 8 mm in diameter were punched on the plates. About 0.5 ml of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37ºC for 24 h. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

RESULT AND DISCUSSION

The results obtained showed that full concentration of ethanolic extracts of *Moringa oleifera*, *Musa paradisiaca*, *Azardiratica indica*, *Cynodon dactylon, Alternanthera sessilis, and Anisochilus carnosus* had inhibitory effects on one of the four tested microorganisms as represented in Tables. Table 1 shows that the full concentration of the ethanolic extracts had mildly active inhibitory effect only on *Escherichia coli*.

The ethanolic extracts of *Moringa oleifera*, *Musa paradisiaca*, *Cynodon dactylon, Alternanthera sessilis, and Anisochilus carnosus* had no inhibitory effects on other microoragnisms. The mean zone of inhibition was found to be 12 mm for that of *Escherichia coli*. However, in this study, the ethanolic extract of *Azardiratica indica* had mild inhibitory effects on the *Klebsiella pneumoniae* and *Bacillus subtilis.*

While the aqueous extract of the same concentration showed no inhibitory effects on the tested microorganisms except *Azardiratica indica* which showed mild inhibitory activity against *Escherichia coli* in Table 2. No doubt, several studies have been conducted in the past three decades that focused on the antimicrobial properties of herbs, spices and their derivates such as essential oils, extracts and decoctions (*Kivanc and Akgül,* 1986*; Dorman and Deans,* 2000*; Hsieh et al.,* 2001*; Ozcan and Erkmen,* 2001*; Alma et al.,* 2003).

Some researches report that there is a relationship between the chemical structures of the most abundant compounds in the tested extracts or essential oils and the antimicrobial activity (*Farag et al*., 1989; *Deans and Svoboda*, 1990). Aromatic phenolic compounds which have been found to have antimicrobial properties (*Alma et al., 2003*).

However, the pH of compounds in dilutions also may have modified the results. Thus, for example, anise oil had higher antifungal activity at pH 4.8 than at 6.8, while the oil of Cedrus deudora is most active at pH 9.0 (*Janssen et al.,* 1987) In conclusion, although the exact active components of the extract that showed this effect were not identified, but antimicrobial active plant principles such as flavonoids, alkaloids and tannins were observed in the extract.

CONCLUSION

The ethanolic extracts of various leaves such as *Moringa oleifera* (Murungai) , *Musa paradisiaca* (Bananana), *Azardiratica indica* (Neem), *Cynodon dactylon*(Grass), *Alternanthera sessilis* (Ponnangkani), *Anisochilus carnosus* (Karpooravalli), investigated individually for antimicrobial activity by disc diffusion method and investigated against selected species of *Escherichia coli, Bacillus subtilis, Vibrio cholerae, Klebsiella pneumoniae* to find the inhibitory activities of the microbes. The ethanolic extract of *Azardiratica indica* showed considerably high activity against *Escherichia coli* than other extracts. These results were compared with standard antibiotic Penicillin. But the extract showed higher activity than the given standard antibiotic.

	Cynodon dactylon	*Azardiratica indica*	*Alternanthera sessilis*	*Moringa oleifera*	*Anisochilus carnosus*	*Musa paradisiaca*
E.coli	++	++	++	++	++	++
B.subtilis	--	--	--	--	--	--
K.pneumoniae	--	--	--	--	--	--
V.cholerae	--	--	--	--	--	--

Table-1: Sensitivity of different microorganisms on full concentration of ethanolic extract of various plant leaves

	Cynodon dactylon	*Azardiratica indica*	*Alternanthera sessilis*	*Moringa oleifera*	*Anisochilus carnosus*	*Musa paradisiaca*
E.coli	--	+	--	--	--	--
B.subtilis	--	--	--	--	--	--
K.pneumoniae	--	--	--	--	--	--
V.cholerae	--	--	--	--	--	--

Table-2: Sensitivity of different microorganisms on full concentration of aqueous extract of various plant leaves

++ Active inhibitory in action

-- No inhibitory in action

+ Slightly inhibitory in action

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