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COMPARATIVE EVALUATION OF ANTIMICROBIAL AND ANTIHELMINTHIC ACTIVITY OF AZADIRACHTA INDICA, CURCUMA LONGA & MURRAYA KOINEGII

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Abstract

Microbes are microscopic organisms living with, in and on human beings from the very beginning of the mankind. Microbial diseases rank as number one cause for almost half the death in underdeveloped and tropical countries. One of the greatest accomplishments of the modern medicine has been the development of antimicrobials for the treatment of infectious diseases. The discovery and development of the antibiotics have led dramatic improvement in the ability to treat infectious diseases and is among the major advances of the 20th century.

Many plants which have a use in day to day life are having potent anti microbial activity. So here we aim at comparatively evaluating the antimicrobial activity of *Azadirachta indica, Curcuma longa & Murraya koinegii* and confirming as to which of these plants have a better activity by performing antihelminthic and antimicrobial screening.

Keywords: Antimicrobial, Anti helminthic, Azadirachta indica, Curcuma longa, Murraya koinegii

Introduction

Microbes are microscopic organisms living with, in and on human beings from the very beginning of the mankind. The microbial population includes potential pathogens microorganism are very diverse. Microbial diseases rank as number one cause for almost half the death in underdeveloped and tropical countries. Hence the need to fine safe and highly effective cure for microbial diseases remains a major challenge for modern science even today. One of the

greatest accomplishments of the modern medicine has been the development of antimicrobials for the treatment of infectious diseases. The discovery and development of the antibiotics have led dramatic improvement in the ability to treat infectious diseases and is among the major advances of the 20th century.

An increase in antimicrobial resistance, multi drug resistance amongst common pathogens throughout the world (e. g. Multi drug resistant tuberculosis) due to irrational and overuse of antibiotics, failure to furnish an antibiotic prescription, genetic versatility of microbes and horizontal transfer of resistant genes among bacterial species. All these are diminishing the clinical usefulness of antibiotic. It is estimated that about 5000 to 10,000 natural antibiotics have been isolated and characterized and 50,000 to 1,00,000 analogues have been synthesized till date, but most of them could not be realized for medicinal use due to toxicity, adverse effects or other practical problems.

Initially most of the antibiotics came from streptomyces and other bacteria and fungi, but for some time now, rare microorganisms area used for isolation of novel antimicrobial agents.

Objective and Plan of Work

Objective

To study the anti-microbial and anti helminthic activity of the following plants (leaves extracts):

Azadiractha indica

Curcuma longa

Murraya koinegii.

Plan of work

To produces the extract of the above listed plants using the following three solvents according to their polarity.

Petrolium ether, Chloroform, Ethyl alcohol

The above extracts were subjected to evaluation of anti microbial and anti helminthes activity against gram positive and gram negative bacteria after which there can be a comparison of results.

Methodology

Plant collection:

The plant Azadirachta indica was collected from the campus of Anwar Ul Uloom college of Pharmacy . Before their use they were carefully identified.

Preparation of the powder:

The leaves of the plant were dried over a polythene cover in shade drying method with a help of a fan at 21^0 C room temperature and pulverized using a mechanical grinder. The powder is further passed through a fine mesh sieve to get a fine powder.

Preparation of the extract:

The coarse material (250gm) was extracted separately with different solvents of increasing order of polarity e.g. petroleum ether, chloroform, ethyl alcohol by soxhletion method during which the temperature was maintained between 35-37 ^{0} C. The liquid extracts that were yielded were concentrared under vaccum to get semi solid residue which was our final.

Preparation of the aqueous extract:

The coarse material (250gm) was taken in a round bottom flask with water quantity of 3-5 liters and macerated for about 72 hours the suspension was filtered through a fine muslin cloth. The filtrate was taken in a glass flask and evaporated at 40-50 ^{0} C with continuous agitation to get a semi solid residue.

Source of chemicals:

All the chemicals and solvents used were of analytical grade and procured from the following manufacturer:

Petroleum ether: S.D fine chemicals ltd. Tilak road Hyd-1.

Chloroform: S.D fine chemicals ltd. Tilak road Hyd-1.

Ethyl alcohol: S.D fine chemicals ltd. Tilak road Hyd-1.

Preparation of nutrient agar medium:

Constituents of agar medium:

For bacteria:

Beef extract 10 g

Sodium chloride 5 g

Peptone 10g

Agar 20g

Distilled water 1000 ml.

Procedure:

- The above constituents, beef extract (12g), sodium hydroxide(5g), peptone(10g) were first dissolved in a beaker containing 100ml of distilled water. The volume was then made up to 1000ml and heated up on attaining boiling temperature, agar (20g) was added and heating was continued for 30 min.
- The pH of the medium was adjusted to7.0-7.5, autoclaved at 121^0 C at 15 lb/ sq. inch pressure for 15 minutes for sub culturing of bacterial strains.
- The bacteria were isolated from soil by **serial dilution method**. And spread on the prepared nutrient agar medium by **spread plate method** for the growth of the bacteria according to the dilutions.

Preparation of nutrient broth medium:

Constituents of nutrient broth medium

Beef extract 10 g

Sodium chloride 5 g

Peptone 10g

Distilled water 1000 ml.

Procedure:

The above constituents were mixed and dissolved in a beaker containing 100ml distilled water. The volume was then made up to 1000ml. with distilled water and pH of the medium was adjusted to 7.0-7.5, autoclaved at 121 ^{0} C at 15lb/sq. inch pressure for 15 minutes and used for sub culturing of bacteria.

Results & Discussion:

Table 1: Antihelminthic activity of various extracts.

Sl.No	Drugs used	Type of Extract	Conc. µg/ml	Time Taken for Paralysis (P)min's	Death Time (D) min's
1.	Vehicle(NS)	-	-	-	-
2.	Piperazine Citrate	-	10	20.22±0.27	42.09±49
3.	Murraya koeingii	Aqueous	50	32.50±0.61	65.67±1.05
			100	22.17±0.79	50.77±1.10
4.	Azadirachta indica	Aqueous	50	39.66+1.17	72.03±1.6
			100	31.34±0.05	62.50±1.8
5.	Curcuma longa	Aqueous	50	51.83±6.8	95.30±0.53
			100	45.47±1	78.41±0.95

Table 2: Antimicrobial Screening of Azadiractha indica.

S.no	Dilution of the soil sample	Type of Bacteria	Zone of Inhibition diameter(mm)		
			E1	E2	E3
1.	10^{-1}	Gm +ve	-	14.5	-
2.	10^{-2}	Gm +ve	-	-	-
3.	10^{-3}	Gm -ve	-	-	-
4.	10^{-6}	Gm -ve	-	18.3	-
5.	10^{-7}	Gm -ve	-	14.8	-
6.	10^{-8}	Gm -ve	-	20.2	-
7.	10^{-10}	Gm -ve	-	15.4	-

Table 3: Antimicrobial Screening of Curcuma longa

S.no	Dilution of the soil sample	Type of Bacteria	Zone of Inhibition diameter(mm)		
			E1	E2	E3
1.	10^{-1}	Gm +ve	-	14	-
2.	10^{-2}	Gm +ve	-	-	-
3.	10^{-3}	Gm -ve	-	-	-
4.	10^{-6}	Gm -ve	-	23	-
5.	10^{-7}	Gm -ve	-	-	-
6.	10^{-8}	Gm -ve	-	-	-
7.	10^{-10}	Gm -ve	-	-	-

Table 4: Antimicrobial Screening of Murraya koinegii

S.no	Dilution of the soil sample	Type of Bacteria	Zone of Inhibition diameter(mm)		
			E1	E2	E3
1.	10^{-1}	Gm +ve	-	23	-
2.	10^{-2}	Gm +ve	-	-	-
3.	10^{-3}	Gm -ve	-	-	-
4.	10^{-6}	Gm -ve	-	30	-
5.	10^{-7}	Gm -ve	-	23	-
6.	10^{-8}	Gm -ve	-	-	-
7.	10^{-10}	Gm -ve	-	-	-

Note:

E1: Petroleum Ether

E2: Ethyl Alcohol

E3: Aqueous Extract

Table 5: Antimicrobial Activity of Standard Drug.

Standard Drug	Conc. µg/ml	Bacilus subtilis	Staphylococcus aureus	Pseudomonas areuginosa
		Zone of Inhibition diameter(mm)		
Gentamicin	100	30	20	25
	40	30	16	20
	20	20	17	15
	10	20	14	14

Conclusion

From the table no 2,3 & 4 we can conclude that the ethanolic extract of the three plants proved to have better activity than aqueous and Petroleum ether extract. From table 2 it was confirmed that ethanolic extract of Azadiractha indica is most potent with a zone of inhibition of 14.5mm against the Gm +ve & Gm-ve microbes comparatively than the other ethanolic extracts shown in table 3 & 4 and has good activity when compared with standard the activity of which is in table 5. Ethanolic extract of Curcuma longa in table no 3 showed least activity with a zone of inhibiton of than the other plant extracts and showed minimum zone of inhibition when compared to Azadiractha indica, Curcuma longa & Murraya koinegii. Murraya koinegii ethanolic extract was moderately potent than the other extracts depicted in table 4 and showed fair zone of inhibition when compared to Curcuma longa. When the antihelminthic property was evaluated it was found that aqueous extract at 100µg/ml showed better effective activity (Table1). Murraya koinegii was the most potent against helmenthis with an paralysis time of 22.17±0.79 and death

time of 50.77±1.10 in mins when compared to Azadiractha indica & Curcuma longa which showed paralysis time of 31.34±0.05 & 45.47±1 respectively and death time of 62.50±1.8 & 78.41±0.95 respectively in mins (Table 1).

So it was concluded that ethanolic extract of Azadiractha indica has the most potent antimicrobial activity compared to Curcuma longa & Murraya koinegii. After the evaluation of anti helminthic activity it was found that aqueous extract of Murraya koinegii has the potent anti helminthic activity compared to Azadiractha indica & Curcuma longa.

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