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Screening of antibacterial and anthelmintic potentials of *Tamarindus indica* and *Carica papaya*

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Abstract

The present study was aimed to evaluate the antibacterial and anthelmintic evaluation of methanolic and aqueous extracts of leaves of *Tamarindus indica*, latex of *Carica papaya*. The antibacterial activity was evaluated by cup diffusion method against both gram positive and gram negative bacteria. Methanol extract of *Carica papaya* latex showed maximum activity against *Bacillus subtilis* (IZ: 29±0.35mm; AI: 1.318). The anthelmintic profile of various extracts of leaves of *Tamarindus indica*, latex of *Carica papaya* showed that aqueous extracts of *Tamarindus indica* greater potency to produce paralysis earlier than other extracts.

Keywords: *Tamarindus indica, Carica papaya*, Antibacterial activity, Anthelmintic activity.

Introduction

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Preparations of plants or parts of them were widely used in popular medicine since ancient times and till today the use of Phytomedicine is widespread in most of the world's population.[1] The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in developing countries.[2] The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies.[3, 4] For a long time, plants have been an important source of natural products for human health.

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties.[5] Plants are the cheapest and safer alternative sources of antimicrobials.[6-8] Helminth infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease distressing a large proportion of world's population. The helminthes parasites mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them.[9] The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment to currently

available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases.[10] Hence there is an increasing demand towards natural anthelmintics

Tamarindus indica L., (Tamarind), family, Leguminaceae, is one such widely used medicinal plant. It is found in virtually all tropical climatic regions, from India through Africa to the Caribbean and South America and up to Southern Florida. It is administered to alleviate sunstroke, digitalis poisoning, and alcoholic intoxication. The pulp is said to aid the restoration of sensation in cases of paralysis. Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. Lotions and extracts made from them are used in treating conjunctivitis, dysentery, jaundice, hemorrhoids and various other ailments, because of their antiseptics and vermifuges properties. The fruit shells are burned and reduced to an alkaline ash, which enters into medicinal formulas.

The bark of the tree is regarded as an effective astringent, tonic and febrifuge. Fried with salt and pulverized to an ash, it is given as a remedy for indigestion and colic. A decoction is used in cases of gingivitis and asthma and eye inflammations. Lotions and poultices made from the bark are applied on open sores and caterpillar rashes. An infusion of the roots is believed to have curative value in chest complaints and is an ingredient in prescriptions for leprosy. Tamarind preparations are universally recognized as refrigerants in fevers and as laxatives and carminatives. Alone, or in combination with lime juice, honey, milk, dates, spices or camphor, the pulp is considered effective as a digestive, even for elephants, and as a remedy for bile disorders and for the treatment of scorbutic disease.[11]

Carica papaya belongs to the family Caricaceae. The parts that are usually used include the leaves, fruit, seed, latex, and root. The plant is described as a fast growing, erect, usually unbranched tree or shrub, 7-8m tall with copious latex, trunk of about 20cm in diameter. The plant is also described in a documented property forms and it act as analgesic, amoebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. *Carica papaya* contains many biochemically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain is used in the treatment of arthritis. Papain also finds its usage in treating ulcers and diphtheria where it is helpful in dissolving the membrane. Extract of chymopapain has been used in treatment of ailments related to disc prolapses and slip disc. The leaves of *Carica papaya* is used as soap substitute which are supposed to remove stains. The papain, the proteolytic enzyme has a wealth of industrial uses. It has milk clotting (rennet) and protein digesting properties. Active over a wide pH range, papain is used in medicine, combating dyspepsia and other digestive orders. In liquid preparations, it has been used for reducing enlarged tonsil.[12]

Materials and methods

Collection of Plant materials

The fresh leaves of *Tamarindus indica* Linn. Family Leguminaceae were collected, and it is authenticated by local botanist. The plant leaves were shade dried at room temperature of (32±2) °C and the dried leaves were ground into fine powder using pulverizer. The powdered part was sieved and stored in cellophane bags.

Fresh latex was collected from locally grown *Carica papaya* initially by 4-6 longitudinal incisions were made on the unripe fruit using a stainless steel knife. The exuded latex was allowed running down the fruit and dripping into collecting devices attached around the trunk. The latex was transferred to a glass bottle and stored at cold temperature. The collected latex is dried in the hot air oven in the range of 65°C to 80°C. After the through drying the latex becomes as to light brown color. Dried papaya latex was extracted by maceration method

Extraction

The powdered leaves and the latex were extracted by cold maceration method. The powder was macerated in methanol and water separately for 24 hours. These solvents are chosen by getting non polar components at first and it tends to polar components. The filtrate was distilled and evaporated on water bath up to a semisolid mass and air dried and kept in vaccum desicator and stored at room temperature.

Collection of experimental cultures and animals

Bacterial cultures of Gram- positive bacteria *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3160) and gram – negative bacteria *Escherichia coli* (MTCC 46) are used for screening. All the test strains were maintained on nutrient agar slopes and were sub cultured once in every two-week. The earth worms are collected from water logged soils near Korangi, Tallarevu [M], East

Godavari district, Andhra Pradesh. They are washed with normal saline solution and stored in tyrode solution.

Preparation of Nutrient Agar

The weighed amount of NaCl, peptone, Beef extract are dissolved in 1000 ml of the water, then agar is added slowly on heating with continues stirring until agar is completely dissolved and pH is adjusted to 7.2 to 7.4. This nutrient agar medium is then sterilized by moist heat sterilization method using autoclave at temperature of 120°C at 15 lb pressure maintained for 15 minutes.

Evaluation of antibacterial activity

The antibacterial activity was done by well diffusion method.[13-17] The wells are made by sterile cork borer (5mm) after solidification of the agar medium. The standard and test dilutions are made with DMF as solvent. 100µg/ml solution of each extract was introduced into cups at sterile aseptic conditions. Ciprofloxacin (10µg/ml) solution was used as standard and DMF was used as control. The plates are incubated in refrigerator for diffusion and then transferred to incubator and incubated at 37ºC for 18hrs. The zone of inhibition was measured and recorded. At the end of the incubation period the antimicrobial activity was evaluated by measuring the average of inhibition zones using digital vernier calipers (including disc diameter of 6 mm). Activity index for each extract was calculated by using following formula.

Activity index (AI) = Inhibition Zone of the sample/ Inhibition Zone of the standard

Evaluation of anthelmintic activity

The anthelmintic evaluation of plant extracts was done by using Indian earth worms.[18-20] Earthworms of 5 to 8cms in length and 0.2 to 0.3cms in width were used. Animals were divided into 15groups containing 3 earthworms in each group. Three different concentrations [25mg/ml, 50mg/ml, and 100mg/ml] of all the extracts were prepared. 50ml of the standard drug piperazine citrate having the concentrations of 25mg/ml, 50mg/ml and 100mg/ml were prepared by using 5% DMF in saline solution which was used as control.

Fifteen petridishes of equal sizes were taken and numbered. 50ml of different concentrations of all the extracts were placed in 12 petridishes. Three different concentrations of piperazine citrate were placed in other three petridishes. Control was maintained in one petridish. All petridish were maintains at room temperature. The time of paralysis was noted when no moment of any sort could be observed except when the worms were shaken vigorously and the time for death recorded after ascertaining that worms neither moved when shaken vigorously or when they dipped in warm water (50°C).

Results and Discussion

In the present investigation, methanol and aqueous extracts of *Tamarindus indica*, latex of *Carica papaya* exhibited nearly similar considerable antibacterial activity indicating the suitability of these solvents for dissolving most of the bioactive compounds of the plants. All the extracts highly affected the activity of gram positive and gram negative bacteria indicate the presence of broad spectrum antimicrobial compounds.

Table 1: Anti bacterial activity of different extracts of leaves of *Tamarindus indica*, latex of *Carica papaya*

Test microorganism		Zone of Inhibition				
		***Tamarindus indica* Linn**		***Carica papaya***		**Ciprofloxacin**
		Methanol	Aqueous	Methanol	Aqueous	
		100 µg/ml	100 µg/ml	100 µg/ml	100 µg/ml	10 µg/ml
Bacillus subtilis	IZ (mm)	26±0.46	17±0.44	29±0.35	28±0.36	22±0.82
	AI	1.181	0.772	1.318	1.272	-
Staphylococcus aureus	IZ (mm)	22±0.82	15±0.22	27±0.45	26±0.29	23±0.82
	AI	0.956	0.652	1.173	1.130	-
Escherichia coli	IZ (mm)	28±0.34	25±0.26	22±0.61	19±0.16	26±0.46
	AI	1.078	0.961	0.846	0.730	-

IZ of negative control for each bacteria is 0.0mm. Values are Mean ± SEM; n=3, ($p>0.05$).

All the extracts showed maximum activity against *Bacillus subtilis*. Methanol extract of *Carica Papaya* latex showed maximum activity against all the test microorganisms. When compared to the other extracts Methanol extract of *Carica Papaya* latex showed maximum activity against *Bacillus subtilis* (IZ: 29±0.35mm; AI: 1.318) and aqueous extract of the *Tamarindus indica* showed least activity against *Staphylococcus aureus* (IZ: 15±0.22mm; AI: 0.652).

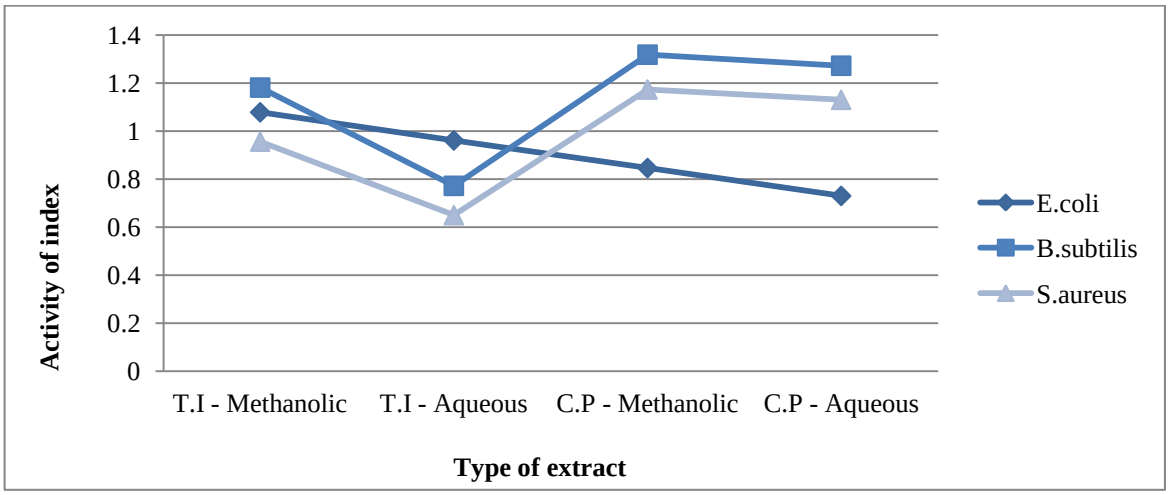

Figure 1: Antibacterial activity of different extracts of *Tamarindus indica* (T.I) leaves and latex of *Carica papaya* (C.P)

The standard drug piperazine citrate that was taken for the study was found to possess anthelmintic activity under the influence of the vehicle containing DMF and normal saline. The control group showed no death and hence it confirms the safety of vehicle.

Table 2: Anthelmintic activity of different extracts of leaves of *Tamarindus indica*, latex of *Carica papaya*

Type of extract	Concentration mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minutes:	
		(P)	(D)
Piperazine citrate	25	32.40 ± 0.06	42.21 ± 0.10
	50	28.30 ± 0.05	38.93 ± 0.05
	100	20.50 ± 0.04	23.68 ± 0.03
T.I - Me	25	19.81 ± 0.04	60.81 ± 0.08
	50	14.01 ± 0.03	45.23 ± 0.06
	100	09.02 ± 0.01	30.58 ± 0.04
T.I - Aq	25	35.00 ± 0.08	44.00 ± 0.07
	50	18.00 ± 0.06	29.00 ± 0.04
	100	07.00 ± 0.04	09.00 ± 0.01
C.P - Me	25	45.50 ± 0.07	62.60 ± 0.09
	50	30.60 ± 0.05	45.80 ± 0.07
	100	27.80 ± 0.02	42.50 ± 0.04
C.P - Aq	25	40.30 ± 0.09	60.30 ± 0.08
	50	29.30 ± 0.06	43.20 ± 0.05
	100	25.60 ± 0.03	40.50 ± 0.01
Control	-	-	-

T.I: *Tamarindus indica*; C.P: *Carica Papaya*; Me: Methanolic extract; Aq: Aqueous extract
Values are Mean ± SEM; n=3 worms in each group $p < 0.05$ is considered as significant when compared with standard drug.

The anthelmintic profile of methanol and aqueous extracts of *Tamarindus indica*, latex of *Carica papaya* shows that aqueous extracts of *Tamarindus indica* greater potency to produce paralysis earlier than other extracts.

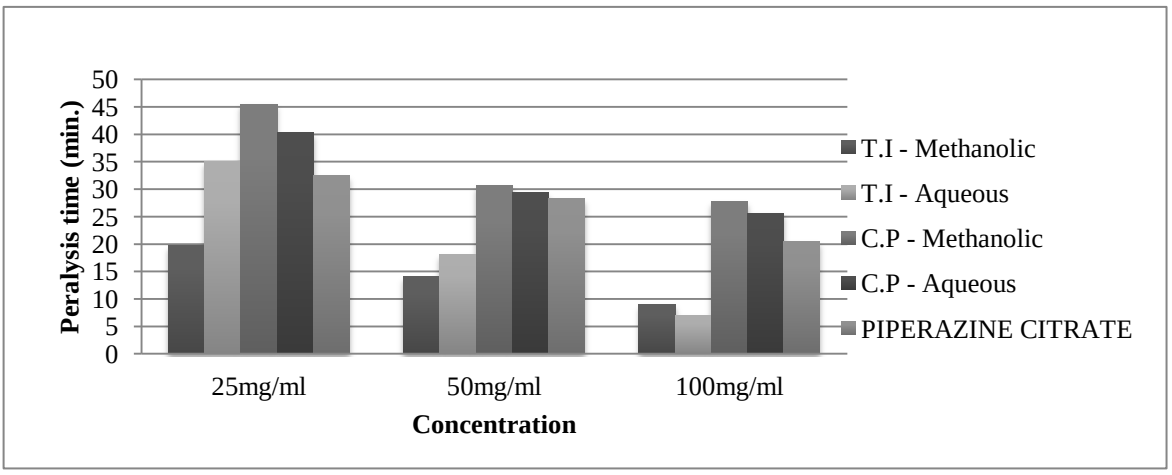

Figure 2: Comparison of paralysis time of different extracts

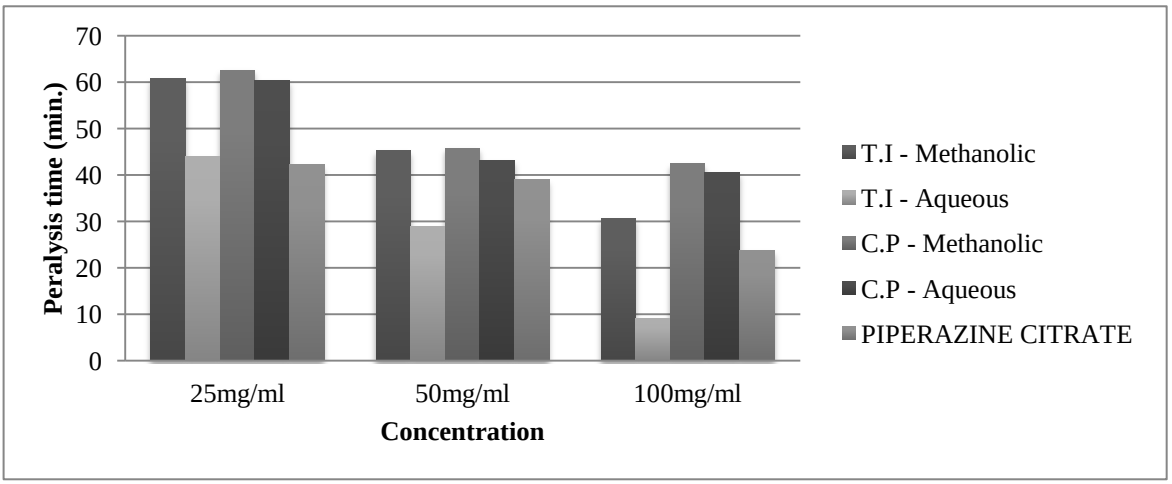

Figure 3: Comparison of death time of different extracts

Conclusion

Though there are a number of antibacterial and anthelmintic drugs available in the market, they produce many side effects. Hence to improve the status of therapy, various traditional plants like *Tamarindus indica*, *Carica papaya* will be much useful. From the results obtained, it was clear that if a detailed research is carried out on the different extracts of *Tamarindus indica*, *Carica papaya* some useful drugs may develop for the treatment of bacterial and helmethic infections. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus, *Tamarindus indica*, *Carica papaya* could become promising natural antimicrobial, anthelmintic agents with potential applications in pharmaceutical industry. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

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