STUDY ON ANTIBACTERIAL ACTIVITY OF CALOTROPIS PROCERA

ORIGNAL Research Article

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Study On The Antibacterial Activity Of Calotropis Procera

ABSTRACT:

Comparative study of plant extracts crude and aqueous, methanolic and ethanolic with antibiotics, provide evidence that calotropis procera extracts has the similar antibacterial activity as these antibiotics against test pathogens i.e. Salmonella typhi and E.coli. The analysis of antimicrobial activity of aqueous, methanolic and ethanolic extract of leaves and flower of *Calotropis procera* was carried out in disc method and also determined MIC value at 600nm through optical density using spectrophotometer. The zone of inhibition produced by extracts was examined and compares it with zone produced by antibiotics. The effect exhibited by ethanolic extract of leaves and flower was significantly greater than the aqueous and methanolic extract of leaves and flower. Crude extracts i.e. latex, leaves, fruit and flower crude extracts. Among them, flower crude extracts shows similar zone of inhibition to test anitibiotics.while in MIC value, we made different concentration of extracts and antibiotics.i.e for crude we made 25%, 50%.75% and 100% concentration of crude juice and for the aqueous, methanolic and ethanolic we made 0.5mg/mL,1mg/mL,2mg/mL,3mg/mL,4mg/mL, 5mg/mL and 6mg/mL in Dimethyl salfoxide, and same antibiotics concentration. Aqueous leaves extracts show MIC at 0.5mg/ml against E.coli while against Salmonella it shows MIC at 1mg/ml... We also determine phytochemical analysis for presences of different compounds in crude extracts. The obtained results provide a support for the use of *Calotropis procera*, in traditional medicine and suggest its further advance investigation.

***Keywords*:** Antimicrobial, *Calotropis Procera, Crude* extracts salmonella *typhi, Escherichia coli.*

INTRODUCTION:

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Different plants are used as medicinal purposes for long time. The folk and traditional medicinal system uses the plant material for the treatment of various diseases. It has been proved that plants are one of the major sources of drug discovery and development. Plants are reported to have anticancer, antimicrobial, ant diabetic, anti inflammation, antioxidant properties (Gaurav Kumar et al., 2010). Calotropis procera commonly known as milk weed (or) swallow wart is a glabrous or hairy laticiferous shrub or small tree found in subtropical Asia and tropical and Africa (Perwez Alam et al., 2008). Traditional doctors in West Africa have claimed to have successfully used the plant to cure many diseases. In the traditional Asian medical system, it has been used for bronchitis, pain, asthma and tumors. The plant is also known for its toxic properties that include dermatitis, iridocyclites and acts like a poison and produces lethal effects (Vadlapudi Varahalarao et al., 2009). The latex of Calotropis procera extract is easily available and is used in medicine for treatment of many diseases. It is used as wound healing agent, anti-diarrheas, anti inflammatory, and anti- rheumatism agent. It is also used against malaria and skin infection (Sammer H Qari et al., 2008). The milky latex and flowers were considered to improve digestion, Catarrh and increases appetite (Oudhia, 2001). In this report, we provide new information on the antibacterial activities of Calotropis procera using known bacterial pathogens as test organism. It is also used by traditional medicine practitioners in Gwari communities for the treatment of ring worms. (Kuta, 2008). Various pharmaceutical companies have produce a number of new antibacterial drugs in the last ten years, resistance to these drugs by bacteria has increased and has now become a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento et al. 2000).the bioactive plant extract is a new concept and has been recently reported (Aburjai et al. 2001).

Main objective of our work is to examine the antibacterial activity of CALOTROPIS PROCERA and to compare its antibacterial potency with antibiotics.

MATERIALS AND METHODS:

Collection and identification of plant materials

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Fresh leaves .Flower Fruit and latex of Calotropis procera were collected from the D.I.KHAN city and maintained in the Microbiology laboratory GOMAL UNIVERSITY, DERA ISMAIL KHAN

Extraction of plant materials:

The plant material (leaves and flower of Calotropis procera) was air dried at room temperature for 20 days after which it was grinded to a fine powder. The extracts of ethanol, methanol and water were prepared by soaking 100g each the dry powdered plant materials in 1 litter of ethanol, methanol and water at room temperature for 4 days with continuous stirring. The extracts were filtered, through a Whitman filter paper and than placed in water bath.

Test Microbe:

Test organisms used were**,** E. coli and salmonella typhi.

Morphological and biochemical characterization of the isolates:

Morphological and biochemical characterization of bacterial strain was tested in laboratory.the presences of colonies was confirmed by salmonella shagella agar,oxidase test and indole test.

Phytochemical screening:

Phytochemical analysis of all crude extracts was carried out according to the methods described by Ayoola G A (2008).

Antibacterial activity:

The antimicrobial activities of water, methanol and ethanol extracts were determined by using disc diffusion method (Kareem SO, Akpar I, and Ojo OP, 2008, Koshyphilip, Sri Nurestri Abd Malek, Wirakarnain Sani, Sim Kae Shin, Saravana kumar, 2009.)

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Disc diffusion:

The nutrient agar plates seeded with bacterial test organisms were allowed to solidify and placed a 6mm filter paper disc, soaked with test extract. The plates were incubated at 37ºC for 48hrs. (Omenka and Osuoha2000).

Determination of minimum inhibitory concentration (MIC):

MIC is the lowest concentration of an antimicrobial that inhibit or kills the visible growth of microorganism.MIC is gernally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms. For crude extracts four different concentrations i.e. 100%.75%, 50%, 25% were prepared for each of fresh Calotropis procera juices. For antibiotics and ethanolic, methanolic and aqueous extracts eight test tubes of different concentration i.e. 0.5mg/mL,1mg/mL,2mg/mL,3mg/mL,4mg/mL, 5mg/mL and 6mg/mL in Dimethyl salfoxide were prepared. The nutrient broth was prepared. From the broth 9ml broth was added in test tube.

Then 1ml of each concentration of crude and powdered ethanolic, methanolic, aqueous extract and antibiotics was added into respective test tube. After this step 0.1 ml test pathogen suspension were inoculated into respective labeled test tube. After inoculation, the test tubes were kept at shaking incubator for overnight at 37oc and results were observed in the form of turbidity and O.D were observed at 600nm on U.V spectrophotometer.

RESULT AND DISCUSSION:

The phytochemical screening of the studies showed the presence of terpenoids, saponins, tannins alkaloids, cardiac glycosides, phenol and tannin, steroids, protein, acidic compounds in crude flower extracts. The crude extract of Calotropis procera leaf showed the absence of steroids and alkaline reagent. The crude fruit and latex extracts of Calotropis procera showed the absence of phenol and tannin (Table1). The antimicrobial activity showed that the leaves of Calotropis procera have bactericidal effects on pathogenic microorganisms .Among the crude extracts (both aqueous and ethanolic) of Calotropis procera flower (aqueous) gave the widest zone of inhibition 32±5mm against E. coli at 75% crude extracts which was nearly similar to zone produced by antibiotic doxycyline and vibramycine was 35±2mm and volosef 33±5 at

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20mg/ml. The crude flower and Fruit (aqueous) gave the widest zone of inhibition 24±5 mm against Salmonella typhi at 75% (Flower) and (Fruit) 25% which was nearly similar to zone produced by antibiotic velosef at 5mg/ml 27±3mm, doxycycline at 5mg/ml gave 29±1mm and vebromycine at 10mg/ml gave 31±1mm

and among Powdered plant material (Leaves and Flower) was extracted with ethanol, methanol and water, which were used in 5mg/ml, 10mg/ml and 20mg/ml respectively, high zone 29±4mm was shown by methanolic leaves extract at 20mg/ml against E. coli and while against Salmonella typhi, ethanolic leaves extracts gave 29±2mm at 20mg/ml.on the other hand ethanolic flower extract gave 34±0 mm at 2omg/ml against salmonella typhi and methanolic flower extracts shows 23±3mm against E. coli at 20mg/ml (Table 2 to 6) while antibiotic velosef shows 29±2mm at 10mg/ml against E.coli and Doxycycline show 34±3mm at 20mg/ml against salmonella typhi nearly equal to our extracts

In determining the minimum inhibition concentration, we examine different results. Crude latex and flower shows MIC at 25% against E.coli while leaves and fruit show on 75%, while against salmonella typhi latex show MIC on 100%.flower on 50%.leaves on 75% and fruit on 25% when diluted in water. but when we dilute crude extract in ethanol result were different, crude flower, leaves and fruit show MIC against both the pathogen at 25% dilution of crude extract white latex show variation against E.coli it show MIC at 50% and against salmonella at 100%.

Now for powdered ethanolic .methanolic and aqueous leaves extracts, MIC value was 0.5mg/ml against E.coli and 1mg/ml against salmonella typhi.while for powdered ethanolic .methanolic and aqueous Flower extracts, MIC value was different. Against salmonella typhi, aqueous extract show MIC at 3mg/ml.methanolic at 1.5mg/ml and ethanolic extracts shows MIC at 1mg/ml.ang against E.coli MIC value of aqueous extract was 1.5mg/ml, for ethanolic MIC was 4mg/ml and for methanolic MIC was 1mg/ml (Table 7 to 11).

While for antibiotics MIC value of Doxycycline against E.coli was 0.0685 and salmonella tyhpi was 0.0670 at 0.5mg/ml, MIC value of vibramycine against E.coli was 0.0866 at 6mg/ml and salmonella tyhpi was 0.0647 at 1mg/ml, similarly MIC value of velosef was 0.0782 against E.coli at 5mg/ml and salmonella tyhpi was 0.0629 at 1.5mg/ml.

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Finally the results agree with the use of Calotropis procera in treatment of different diseases because of its bactericidal effects. Finally the results established a good support for the use of Calotropis procera in traditional medicine for many diseases.

CONCLUSION:

The phytochemical analysis reports the presence of antimicrobial active agents such as alkaloids, flavonoids, tannins, saponins etc. The flavonoids and tannins are phenolic compounds and plant phenolics are major group of compounds that act as primary anti oxidants. The present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human and plant systems and has same antibacterial activity as antibiotics shows. The phytochemical analysis revealed that the active principle responsible for the activity is a phenolic compound. So the result established a good support for the use of Calotropis procera in traditional medicines.

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TABLES

TABLE 1: PHYTOCHEMICAL ANYLISIS OF CRUDE EXTRACTS

TEST	L(1)	F(2)	F(3)	L(4)
PHENOL & TANNIN	+	+	-	-
STEROID	-	+	+	+
CARDIC GLYCOSIDE	+	+	+	+
TERPENOIDS	+	+	+	+
SAPONINS	+	+	+	+
PROTEIN	+	+	+	+
ACIDIC COMPOUND	+	+	-	-
CARBOHYDRATES	+	+	+	+
ALKALINE REAGENTS	-	+	-	+

(+ = present, - = absent)

L(1)=LEAVES ,F(2)=FLOWER,F(3)=FRUIT,L(4)=LATEX

TABLE 2: Zone of inhibition of crude plant extracts against E.coli

Extracts	Aqueous				Ethanolic		
Dilution	100%	75%	50%	25%	75%	50%	25%
Latex	17 ±1	19 ±1	16 ±1	14 ±0	11 ±2	11 ±0.5	9 ±1
Leaves	15 ±0	15 ±1	17 ±1	20 ±4	9 ±1	13±1	14±0
Flower	18 ±2	32±4.5	26 ±3	16 ±2	13±1	12±1	11±1
Fruit	11±1.5	10 ±1	9 ±1	8 ±0	10±1	8±0.5	8±0

TABLE 3: Zone of inhibition of crude plant extracts against salmonella typhi

Extract	Aqueous				Ethanolic		
Dilution	100%	75%	50%	25%	75%	50%	25%
Latex	18±1	18±0.5	14±0	11±0	12±1	12±0	8±1
Leaves	15±1	12±1	16±0.5	19±0	13±1	13±2	14±1
Flower	20±2	24±5	17±1	16±1	20±1	12±1	7±1
Fruit	19±1	13±0	16±3	24±5	14±1	15±3	21±4

TABLE 4: Zone of inhibition of Leaves extracts

MICROBE	E.coli			Salmonella typhi		
Dilutions	5mg\ml	10mg\ml	20mg\ml	5mg\ml	10mg\ml	20mg\ml
Aqueous	16+2	17±5	19±4	16±4	21±1	26±1
Methanolic	21±1	24±0	29±4	17±2	19±2	24±2
Ethanolic	19±2	20±3	29±2	23±3	27±2	29±2

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TABLE 5: Zone of inhibition of Flower extracts

MICROBE	E.coli			Salmonella typhi		
Dilutions	5mg/ml	10mg/ml	20mg/ml	5mg/ml	10mg/ml	20mg/ml
Aqueous	10±0	12±1	14±1	16±1	19±0	22±2
Methanolic	15±0	17±2	23±3	17±0	20±1	21±3
Ethanolic	12±0	14±1	16±0	29±1	32±1	34±0

TABLE 6: Zone of inhibition of Antibiotics

MICROBE	E.coli			Salmonella typhi		
Dilutions	5mg/ml	10mg/ml	20mg/ml	5mg/ml	10mg/ml	20mg/ml
Doxycycline	33±3	35±2	37±1	29±1	32±3	34±3
vebromycine	26±1	32±3	35±2	24±3	31±1	36±1
velosef	26±1	29±2	33±5	27±3	33±3	36±3

Table7. MIC Trough O.D AT 600 of CRUDE EXTRACTS WITH AQUEOUS DILUTIONS

MICROBE	E.coli				Salmonella typhi			
Dilutions	100%	75%	50%	25%	100%	75%	50%	25%
Latex	0.5704	0.4192	0.4994	***0.3377***	***0.3508***	0.3899	0.3841	0.5090
Flower	0.9027	0.7178	0.7068	***0.3624***	0.7974	0.8607	***0.6714***	0.6732
Fruit	0.6866	***0.5413***	0.5754	0.6618	0.8712	0.8027	0.8035	***0.5252***
Leaves	0.9563	***0.7072***	0.7464	0.9684	1.0183	***0.8135***	1.0255	0.9452

Table8: MIC through O.D AT 600 of CRUDE EXTRACTS WITH ETHANOLIC DILUTIONS

MICROBE	E.coli				Salmonella typhi			
Dilutions	100%	75%	50%	25%	100%	75%	50%	25%
Latex	0.5704	0.9174	***0.4614***	0.5522	***0.3508***	0.6900	1.7442	0.4913
Flower	0.9027	0.7645	0.5050	***0.4369***	0.7974	0.7200	0.3809	***0.1663***
Fruit	0.6866	0.9474	0.3164	***0.1891***	0.8712	0.8144	0.3623	***0.1872***
Leaves	0.9563	0.7668	0.2080	***0.1665***	1.0183	1.0462	0.2124	***0.1304***

. Table: 9 MIC through O.D AT 600 of *LEAVES EXTRACTS* against E.coli

MICROBE	E.coli							
Dilutions	0.5mg/ml	1mg/ml	1.5mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	6mg/ml
Aqueous	***0.3009***	0.3808	0.3613	0.3560	0.4109	0.3820	0.4200	0.3503
Methanolic	***0.3179***	0.4584	0.4030	0.8233	0.8975	0.5351	0.6134	0.5468
Ethanolic	***0.1636***	0.6008	0.2440	0.3427	0.4628	0.4006	0.3968	0.6508

Table10. MIC through O.D AT 600 of *LEAVES EXTRACTS* against salmonella typhi

MICROBE	Salmonella typhi							
Dilutions	0.5mg/ml	1mg/ml	1.5mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	6mg/ml
Aqueous	0.3218	***0.3170***	0.3638	0.4285	0.4050	0.3428	0.3723	0.4687
Methanolic	0.3721	***0.3580***	0.5060	0.7636	0.9026	O.5314	0.6538	0.6504
Ethanolic	0.1805	***0.1290***	0.1349	0.1954	0.2318	0.2836	0.3087	0.3606

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Table11. MIC through O.D AT 600 of *FLOWER EXTRACTS* against E.COLI

MICROBE	E.coli							
Dilutions	0.5mg/ml	1mg/ml	1.5mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	6mg/ml
Aqueous	0.2215	0.0901	***0.0784***	0.0866	0.0938	0.1022	0.0968	0.0931
Methanolic	0.3106	0.2913	0.2635	0.3165	0.3079	***0.2062***	0.3338	0.3115
Ethanolic	0.2638	***0.1635***	0.2897	0.3171	0.3742	0.3597	0.2471	0.4591

Table12. MIC through O.D AT 600 of *FLOWER EXTRACTS* against salmonella typhi

MICROBE	Salmonella typhi							
Dilutions	0.5mg/ml	1mg/ml	1.5mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	6mg/ml
Aqueous	0.1968	0.1633	0.1677	0.1236	***0.0826***	0.1253	0.2020	0.0925
Methanolic	0.3486	0.3637	***0.2740***	0.3722	0.3929	0.3968	0.5013	0.5193
Ethanolic	0.2331	***0.2309***	0.2477	0.2705	0.3962	0.3931	0.2740	0.5225

Table13. MIC through O.D AT 600 of *ANTIBIOTICS against* E.COLI

MICROBE	E.COLI							
DILUTION	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	5 mg/ml	6 mg/ml
DOXYCYCLINE	***0.0685***	0.0853	0.0757	0.0738	0.0732	0.0751	0.0762	0.0891
VIBROMYCINE	0.0946	0.1328	0.1012	0.1067	0.1268	0.1319	0.3222	***0.0866***
VELOSEF	0.3375	0.4852	0.4087	0.1964	0.4540	0.2488	***0.0782***	0.0786

Table14 .MIC through O.D AT 600 of *antibiotics* against salmonella typhi

MICROBE	Salmnella typhi							
DILUTION	0.5mg/ml	1mg/ml	1.5mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	6mg/ml
DOXYCYCLINE	***0.0670***	0.0697	0.0718	0.0770	0.0780	0.0777	0.0868	0.0806
VIBROMYCINE	0.1312	***0.0647***	0.0712	0.0714	0.0812	0.0772	0.0939	0.0963
VELOSEF	0.0861	0.0666	***0.0629***	0.0669	0.0784	0.0663	0.0706	0.0713

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