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AN EVALUATION OF ANTIBACTERIAL POTENTIAL OF MEDICINAL PLANT *CROTON BONPLANDIANUM* AGAINST SOME PATHOGEN ISOLATED FROM COMPLICATED URINARY TRACT INFECTIONS (UTI): ALTERNATIVE APPROACHES OF CONVENTIONAL TREATMENT

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ABSTRACT

Medicinal plants produce several antibacterial compounds. These plants are well known for their medicinal value and are widely used in community for the treatment of various diseases. It was a study to investigate the Antibacterial Activity of *Croton bonplandianum* Plants Used against UTI Causing Pathogens. Bacteria were isolate from the UTI infected patient and characterized by using microscopic, staining, morphological and biochemical methods. Organic compounds with plant were extracted using a various concentrations and these extracts were than to check their antibacterial activity against the bacteria isolated from UTI infected patients and the zone of inhibition were compared with the zone of inhibition of standard antibiotics. Results from the present study showed that the extracts of *C. bonplandianum* had more antibacterial activity compared to antibiotics. Hexane, Ethyl Acetate, Ethanolic and Chloroform Extracts of *C.bonplandianum* were used for studying antibacterial activity by agar well diffusion assay and Minimum inhibitory concentration method. Among the four extracts used, highest antibacterial activity was recorded with ethyl acetate extract on *E.coli* and least against *Staphylococcus haemolyticus* with diameter of inhibition zones (DIZ) of 2.96 ± 0.57 and 2.79 ± 0.57 mm respectively and at parallel way among four extract highest antibacterial activity recorded with UTI cure antibiotic with Ciprofloxacin on *Enterobacter aerogenes* and least

against *E.coli* with diameter of inhibition zones of 2.66±0.23 and 2.0±0.23mm respectively. Preliminary phytochemical analysis of the plant parts revealed the presence of active compounds such as phenolics, tannins, alkaloids and flavonoids. This study highlights the need to exploit the antibacterial potential of these plants for development of new antibiotics.

KEYWORDS: *Croton bonplandianum, Staphylococcus haemolyticus, E.coli.*

INTRODUCTION

A urinary tract infection (UTI) is an infection in any part of urinary system like kidneys, ureters, bladder and urethra. Most infections involve the lower urinary tract like the bladder and the urethra. Increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs.[1] Infection limited to your bladder can be painful and annoying. However, serious consequences can occur if a UTI spreads to kidneys. Bacteria are ubiquitous pathogens causing various types of infections including urinary tract infections[2], nosocomial bloodstream infections[3], wound infections[4], brain abscess[5], asthma[6], community-acquired pneumonia[7] and skin infections.[8] These infections including urinary tract infections can result in fatal consequences if they are not treated properly or are left untreated. To treat all kinds of bacterial infections antibiotics are used worldwide; however, bacteria are gradually becoming resistant against these antibiotics. Furthermore, during recent years, misuse of antibiotics is calling for an accelerated search for novel antibacterial therapeutic agents.[9] Due to the indiscriminate use of antimicrobial drugs, the microorganisms have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases.[10] In addition to this problem, antibiotics have several adverse effects which include hypersensitivity, depletion of beneficial gut flora, immune suppression and allergic reactions.[11] Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. The present investigation represents a preliminary screening on various plant extracts for the isolation and identification of biologically active compounds having antimicrobial activity. Most of anti-microbial drugs are natural products derived from plants.[12] The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of this family include *E.coli, Klebsiella, Enterobacter* and *Proteus*. Also Gram positive *Staphylococcus* sp. plays a role in the infection (Kunin, 1997). *E.coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections (Iroha, 2009). Nowadays, drug resistance is a huge

growing problem in treating infectious diseases like malaria, tuberculosis, diarrheal diseases, urinary tract infections etc. According to Goldman and Huskins (Goldman and Huskins, 1997) the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance, which became a major health problem worldwide. In the last 3 decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing UTIs (Tenver and McGowanJr, 1996; Kurutepe *et al*., 2005). Essential oils and extracts of certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods.[13] The synergistic effect of the mixture of phytochemicals play important role to use plant extracts as antimicrobial agents.[14] It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels.[15] The antimicrobial and other biological activities of the essential oils varied depending upon the origins and cultivars.[16] Treatment of UTI depends upon the status of the patient. Especially, in case of pregnant females one has to recommend drug keeping its safety in mind. Also, the treatment of urinary tract infections is increasingly becoming difficult because of the multidrug resistance exhibited by the causative organisms. Isolates causing complicated UTI show greater drug resistance as compared to those isolated from uncomplicated UTI5. The increase in incidence of resistant organisms such as extended spectrum β- lactamase producing strains of *Escherichia coli* and *Proteus vulgaris* and methicillin resistant *Staphylococcus* species among clinical isolates over the past few years has resulted in limitation of currently available therapeutic options.[17,18,19] This situation has forced the researchers to search for new antimicrobial substance from various sources including medicinal plants.[20] Medicinal plants represent a rich source of antimicrobial agents. The effects of plant extracts on bacteria have been studied by a large number of researchers in different parts of the world. The main purpose of the current study was to assess the inhibitory activities leaf extract (Ethyl Acetate, Chloroform, Hexane, Methanol, Benzene) of a medicinal plant against some human pathogens that cause urinary tract infections. The Medicinal plant included in study is *Croton bonplandianum*. The present study mainly focused to determine activity against multidrug resistance bacteria isolated from UTI.

Phytochemical aspect of Plant extract (*Croton bonplandianum)*

The genus *Croton* contains diverse types of biomolecules. Terpenoids are the predominant secondary metabolite constituents in the genus, chiefly diterpenoids, which may belong to the cembranoid, clerodane, neoclerodane, halimane, isopimarane, kaurane, secokaurane, labdane, phorbol and trachylobane skeletal types. Triterpenoids, either pentacyclic or steroidal, have frequently been reported from *Croton* species. Volatile oils containing mono and sesquiterpenoids, and sometimes also shikimate-derived compounds are not rare in the genus. Several species have been reported as sources of different classes of alkaloids, a fact that enhances considerably the importance of the genus from the medicinal point of view. Phenolic substances have frequently been reported, among which flavonoids, lignoids and proanthocyanidins predominate. (Salatino *et al.*, 2007) Several species of *Croton*, containing pro-anthocyanidins and/or alkaloids have a red sap. The latter may be taspine or some of several benzyl isoquinoline like compounds. Diterpenes are very common in *Croton*, corresponding to clerodanes, cembranoid, halimanes, kauranes, labdanes, phorbol esters, trachylobanes and sarcopetalanes. Some species are aromatic due to the possession of volatile oils. Representatives of new classes of compounds (phenylbutanoids, glutarimide alkaloids, sarcopetalane diterpenes) have been isolated from *Croton* species. While laticifers have been described in *Croton* species, so far there are no anatomical studies about secretory structures of volatile oil. Few studies about flavonoids have been carried out with *Croton* species. Chemical affinities are apparent in the genus, grouping species with (i) kauranes and/or labdanes, (ii) trachylobanes and (iii) alkaloids. Pharmacological assays have frequently corroborated the traditional uses of *Croton* species. A great part of pharmacological assays with *Croton* substances dealt with the clerodane trans-dehydrocrotonin. (Salatino *et al*., 2007) In 2010 A new flavone, named crotoncaudatin, was isolated from the stems of *Croton bonplandianum* Geisel. var. tomentosus Hook., together with nine known analogues: 3,5,6,7,8,3′,4′-heptamethoxyflavone, tangeretin, nobiletin, 5,6,7,4′-tetramethoxy-flavone, sinensetin, kaempferol, tiliroside, kaempferol-3-O-rutinoside and rutin. (Zou *et al.*, 2010) Ethanol extract of the leaves of *Croton steenkampianus* contains a indanone derivative and two diterpenoids together with three flavonoids. (Adeboye *et al.*, 2008) Some antimicrobial compounds like acetyl aleuritolic acid, stigmasterol, *β*- sitosterol, campesterol, *β*-sitosterol-*O*-glucoside, sonderianin, catechin and gallocatechin was isolated from methanolic extract of *Croton urucuruna*. (Marize *et al.*, 1997) Phytochemically the plant has been reported to contain Rutin (C18 H36 O19) as main constituent together with crotsparinine, crotosparine and its methyl derivatives aphorbol which play a key role in wound healing. (Divya *et al.*,

2011) Apart from these *Croton bonplandianum* is a good source of Steroids, Unsaturated steroids, Phenolics, Alkaloids. It also contains Flavons and flavonols, Cardinolids, Leuco antho –cyanin and Flavonoids. (Kothale *et al.,* 2011). The plant also contains another two groups of compounds viz. Terpenoids and Glycosides along with flavonoids and alkaloids. The spent residue obtained after biocrude extraction of *Croton bonplandianum* is rich in biopolymers, such as cellulose, hemicellulose and lignin. Oil and ethanol can be obtained from this. (Sharma *et al.*, 1990) Jeeshna *et al.*, (2011) studied on the potentiality of different solvent to extract different group of compound from *Croton bonplandianum* and they showed Methanol was much more effective to extract Alkaloids, Flavonoids, Glycosides, Steroids, Phenols, Tannins, Saponins and Resins followed by Acetone, Chloroform and Petroleum ether. Although chloroform fraction does not contain alkaloids and saponins. (Jeeshna *et al.*, 2011) Some isolated triterpenoid from the root of *C. bonplandianum* are 3-hydroxyurs-12,15-dien of ursane skeleton, oleanolic acid, ursolic acid and sitosterol. (Ghosh *et al*., 2013). Alkaloid isolated from extracts of *Croton bonplandianum* 3-methoxy-4,6-dihydroxymorphinandien-7-one, and norsinoacutine.(Tiwar *et al*.,1981).

MATERIALS AND METHOD

Collection of UTI infected culture

First of all, microorganisms present in urine samples of UTI infected patients were collected from Tarakeswar Rural Hospital (Tarakeswar, Bajitpur, Hooghly). Mid stream urine sample was collected in sterile wide mouthed containers.

Collection of Plant

The plant *Croton bonplandianum* is collected from the rural area of Champadanga (Champadanga, Hooghly).

Taxonomical Position of *Croton bonplandianum*

Kingdom : Plantae

Subkingdom : Tracheobionta

Infrakingdom : Streptophyta

Superdivision : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Malpighiales

Family : Euphorbiaceae

Subfamily : Crotonoideae

Tribe : Crotoneae

Genus : *Croton*

Species : *Croton bonplandianum*

Preparation of Plant extract

At first the plant part (leaf) was washed with distilled water, dried in shade, grinded to fine powder and stored in airtight containers at room temperature in dark until used. The powdered samples were subjected to extraction by the following method of Gupta *et al*. (2009).

Hexane Extraction

5g of air dried powder of *Croton bonplandianum* leaf extract was mixed with 25ml Hexane to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized glass tubes until use.

Chloroform Extraction

5g of air dried powder of *Croton bonplandianum* leaf extract was mixed with 25ml Chloroform to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized glass tubes until use.

Ethyl Acetate Extraction

5g of air dried powder of *Croton bonplandianum* leaf extract was mixed with 25ml Ethyl Acetate to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized glass tubes until use.

Ethanol Extraction

5g of air dried powder of *Croton bonplandianum* leaf extract was mixed with 25ml organic Ethanol to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized glass tubes until use.

Preparation of UTI infected culture for further identification

Urine sample of UTI infected patient was cultured in the nutrient broth and after it the morphology of organisms were studied with the help of light microscope and shape, size, odour, margin and surface characteristics of bacteria were studied. Gram staining procedure was adopted to differentiate between Gram positive and Gram negative organisms. Selective agar medium was used for further identification. Seven Biochemical tests (Like- Catalase test, Indole test, MR test, VP test, TSI test, Citrate test, Urease test, and Gas production test) studied in secondary identification of organisms.

Testing of Cytotoxic activity

In vitro Cytotoxic activity was performed by spectrophotometer method (Yang et al., 2005). A volume of 0.2 ml of the cell suspension was mixed with 0.8 of the plant extracts (25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37°C in a incubator. The mixture was centrifuged at 1500 rpm for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as Negative and Positive hemolytic controls The level of percentage hemolysis by the extracts was calculated according to the following formula:

$$\% \text{ Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

Here: A_t is the absorbance of test sample.

A_n is absorbance of the control (saline control)

A_c is the absorbance of the control (water control)

Testing of Antimicrobial activity

'Well plate diffusion' method

Muller Hinton agar was use to check antimicrobial activity by well diffusion method. Autoclaved medium was poured in to petriplates in the laminar air flow hood. On cooling the medium within petriplates the microorganism from 24 hrs old broth were spread then wells

were made on the petriplates with the help of stainless steel borer of diameter 6- 8 mm. Five plates were also made for each microorganism and one wall (Two part) made on each plate the entire surface at angle 180^{o}, Two plate was for four type of concentration (300 µg/ml, 25 µg/m; 150 µg/ml, 75 µg/ml) of each extract of *Croton bonplandianum*, Two plate is for same type of concentration (as the leaf extract) of each type of antibiotic (Ciprofloxacin, Tinidazol, Norfloxacin, Metronidazol) and one for Control (by DMSO). Because we have to prove the extracts have large zone of inhibition than those antibiotics by this parallel study. These plates were incubated for 24 - 48 hrs and the diameter of zone of inhibition was measured with the help of scale.

Statistical Analysis

Results obtained were analyzed statistically and values were expressed as Mean ± SD.

RESULTS

After studying the colony morphology on nutrient agar medium, colony morphology was also studied on the selective media. And we obtained each selective organism's colony appeared in each selective media like.

Table 1: Primary Identification of bacteria isolated from UTI infected urine sample.

Name of Selective media	Identified bacteria
Mac conkey	*E. coli* and *Staphylococcus* sp.
EMB	*E. coli*
MSA	*Staphylococcus* sp.
Blood agar	*Proteus* sp.

After this step final identification of bacteria was done on the basis of biochemical testing.

Biochemical Tests

Seven biochemical tests were performed for each organism as given below:

Table 2: Secondary Identification of bacteria isolated from UTI infected urine sample.

SL No.	Catalase	Indole	MR	VP	TSI	Citrate	Ureage	Gas Production	Organism confirmed
1.	+	+	-	+	+	-	-	+	*Escherichia coli*
2.	+	+	+	-	+	+	+	+	*Proteus vulgaris*
3.	+	-	-	-	-	+	-	+	*Enterobacter aerogenes*
4	+	-	+	+	+	-	-	+	*Staphylococcus haemolyticus*

Resulting Antimicrobial Activity

Four organic compounds (Hexane, Ethyl acetate, Ethanol and Chloroform) with grinned leaf of *Croton bonplandianum* were extracted to test the antimicrobial activity on the four different bacteria isolated from urine sample of UTI infected patient with respect to standard antibiotic by the "agar well diffusion method" and the diameter zone of inhibition was measured in mm. Antimicrobial activity of different extract with different concentrations on the different organisms is given as in Table 3(*E.coli*), Table 4 (*Proteus vulgaris*), Table 5 (*Enterobacter aerogenes*), Table 6 (*Staphylococcus haemolyticus.*

Table No. 3: Treatment with Various extract of *Croton bonplandianum* against UTI causing pathogen- *Escherichia coli.*

Concentration (µg/ml)	Average zone (mm) of inhibition of Various extract of *Croton bonplandianum*			
	Hexane	Ethyl Acetate	Ethanol	Chloroform
300	2.4±0.58	2.96±0.57	2.33±0.58	2.06±0.60
150	2±0.58	2.1±0.60	2±0.58	1.87±0.58
75	2.06±0.57	2.16±0.57	1.56±0.57	1.37±0.58
25	2.2±0.57	2.06±0.57	1.43±0.57	1.12±0.57

Table No. 4: Treatment with Various extract of *Croton bonplandianum* against UTI causing pathogen- *Proteus vulgaris.*

Concentration (µg/ml)	Average zone (mm) of inhibition of Various extract of *Croton bonplandianum*			
	Hexane	Ethyl Acetate	Ethanol	Chloroform
300	2.63±0.57	2.82±0.58	2.26±0.58	2.16±0.58
150	2.43±0.58	2.6±0.60	1.43±0.57	1.23±0.58
75	2.1±0.58	2.03±0.57	1.18±0.57	1.16±0.58
25	1.89±0.57	1.9±0.58	1.05±0.57	1.03±0.60

Table No. 5: Treatment with Various extract of *Croton bonplandianum* against UTI causing pathogen- *Enterobacter aerogenes.*

Concentration (µg/ml)	Average zone (mm) of inhibition of Various extract of *Croton bonplandianum*			
	Hexane	Ethyl Acetate	Ethanol	Chloroform
300	2.63±0.58	2.9±0.57	2.16±0.57	1.96±0.57
150	2.0±0.57	2.13±0.57	1.56±0.60	1.46±0.57
75	1.46±0.57	2.04±0.60	1.23±0.60	1.13±0.57
25	1.13±0.57	1.65±0.60	1.06±0.58	1.02±0.57

Table No. 6: Treatment with Various extract of *Croton bonplandianum* against UTI causing pathogen-*Staphylococcus haemolyticus.*				
Concentration (µg/ml)	**Average zone (mm) of inhibition of Various extract of *Croton bonplandianum***			
	Hexane	**Ethyl Acetate**	**Ethanol**	**Chloroform**
300	2.85±0.58	2.79±0.57	2.32±0.58	2.12±0.60
150	2.43±0.58	2.56±0.57	2.03±0.58	2.01±0.60
75	2.12±0.58	2.31±0.60	1.86±0.57	1.73±0.57
25	1.85±0.57	2.03±0.57	1.62±0.58	1.31±0.57

With parallal study we have proved that The antibiotics(Ciprofloxacin, Tinidazol, Norfloxacin, Metronidazol), that cures naturally the UTI desease, have less zone of inhibition as the extracts of *C. bonplandianum* have. The zone of inhibition of those antibiotics given as Table 7(Antibiotic treatment of *E. coli*), Table 8(Antibiotic treatment of *Proteus vulgaris*), Table 9(Antibiotic treatment of *Enterobacter aerogenes*), Table 10 (Antibiotic treatment of *Staphylococcus haemolyticus*).

Table No. 7: Treatment with Various Antibiotics against UTI causing pathogen- *Escherichia coli.*				
Concentration (µg/ml)	**Average zone (mm) of inhibition of Various UTI cure Antibiotics**			
	Ciprofloxacin	**Tinidazol**	**Norfloxacin**	**Metrotinidazol**
300	2±0.23	1.76±0.24	2.13±0.23	2.06±0.24
150	1.63±0.23	1.43±0.25	1.9±0.23	1.83±0.24
75	1.43±0.24	1.2±0.24	1.43±0.23	1.16±0.23

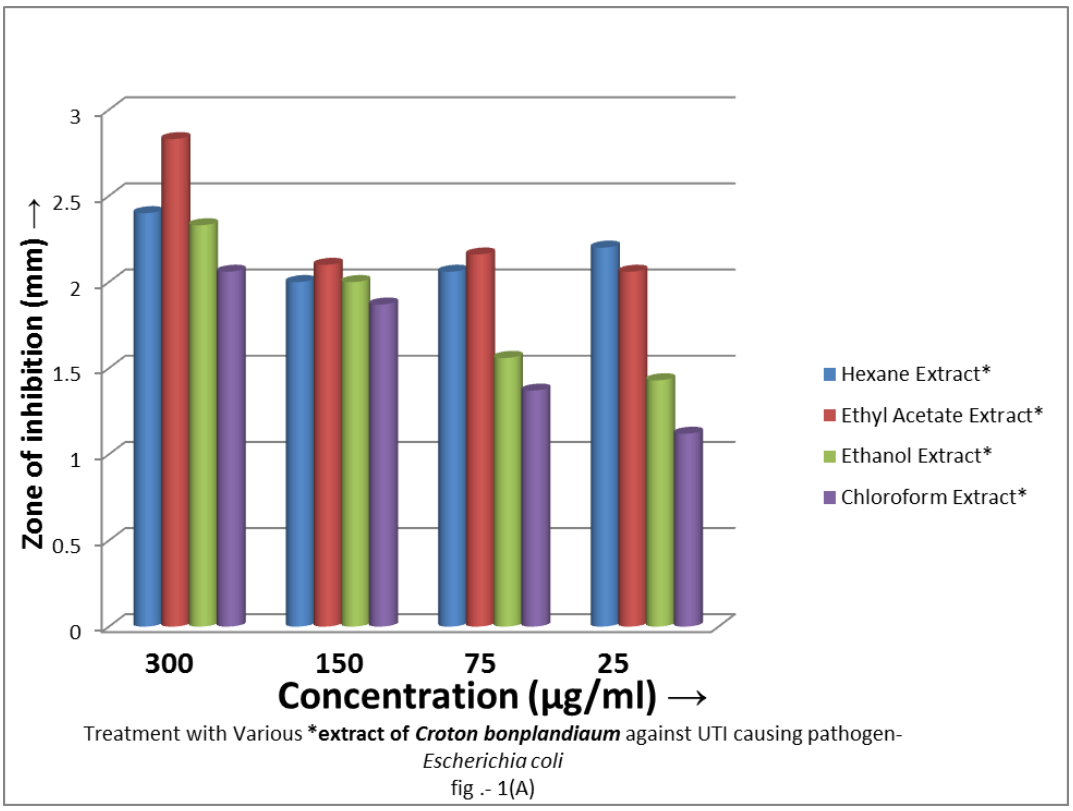
Table no. 8: Treatment with Various Antibiotics against UTI causing pathogen- *Proteus vulgaris.*				
Concentration (µg/ml)	**Avarage zone (mm) of inhibition of Various UTI cure Antibiotics**			
	Ciprofloxacin	**Tinidazol**	**Norfloxacin**	**Metrotinidazol**
300	2.23±0.25	1.9±0.23	1.63±0.25	2.03±0.23
150	2.06±0.24	1.5±0.23	1.45±0.25	1.63±.23
75	1.8±0.24	1.23±0.24	1.24±0.23	1.28±0.23
25	1.53±0.24	1.03±0.24	1.05±0.23	1.01±0.24
25	1.23±0.23	1.06±0.23	1.14±0.23	1.03±0.23

Table no. 9: Treatment with Various Antibiotics against UTI causing pathogen- *Enterobacter aerogenes.*				
Concentration (µg/ml)	**Average zone (mm) of inhibition of Various UTI cure Antibiotics**			
	Ciprofloxacin	**Tinidazol**	**Norfloxacin**	**Metrotinidazol**
300	2.66±0.23	2.3±0.24	2.05	2.14
150	2.0±0.24	1.83±0.23	1.6	1.93
75	1.56±0.23	1.46±0.23	1.4	1.54
25	1.04±0.23	1.2±0.23	1.12	1.16

Table no. 10: Treatment with Various Antibiotics against UTI causing pathogen- *Staphylococcus haemolyticus.*

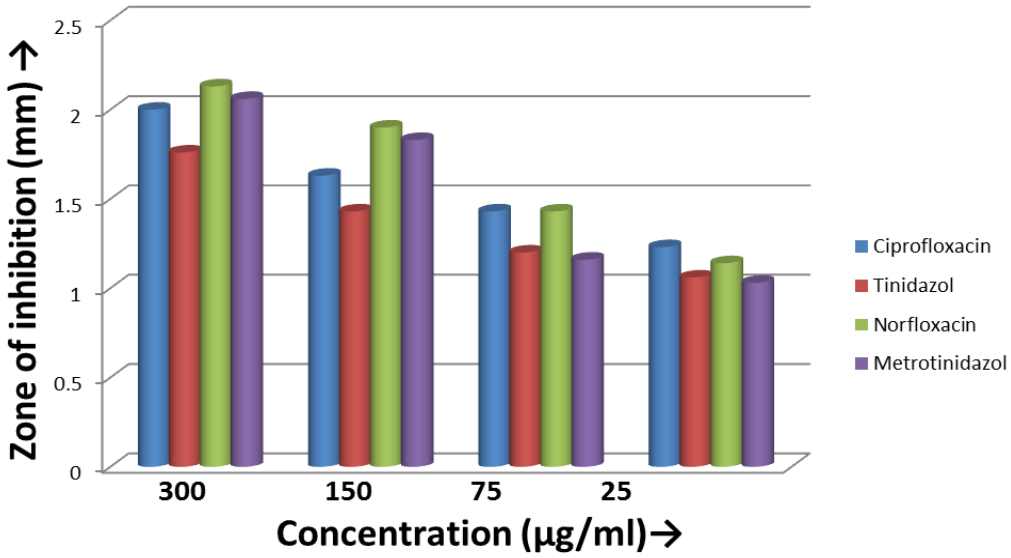
Concentration (µg/ml)	Average zone (mm) of inhibition of Various UTI cure Antibiotics			
	Ciprofloxacin	Tinidazol	Norfloxacin	Metrotinidazol
300	2.64	2.39	2.14	2.03
150	2.02	1.81	1.66	1.69
75	1.34	1.28	1.43	1.42
25	1.01	1.02	1.10	1.06

Here is some another comparing study, that proves the various Extracts of Croton bonaplandium have much antibacterial activity than the antibiotics aganst those UTI causing bacteria. Determination is given below as Figure 1(A), 1(B) and Figure 1 (C) for *E. coli*, Figure 2(A), 2(B) and Figure 2 (C) for *Proteus vulgaris*, Fig 3(A), 3(B) and Figure 3 (C) for *Enterobacter aerogenes*, Figure 4(A), 4(B) and Figure 4 (C)for *Staphylococcus haemolyticus.*



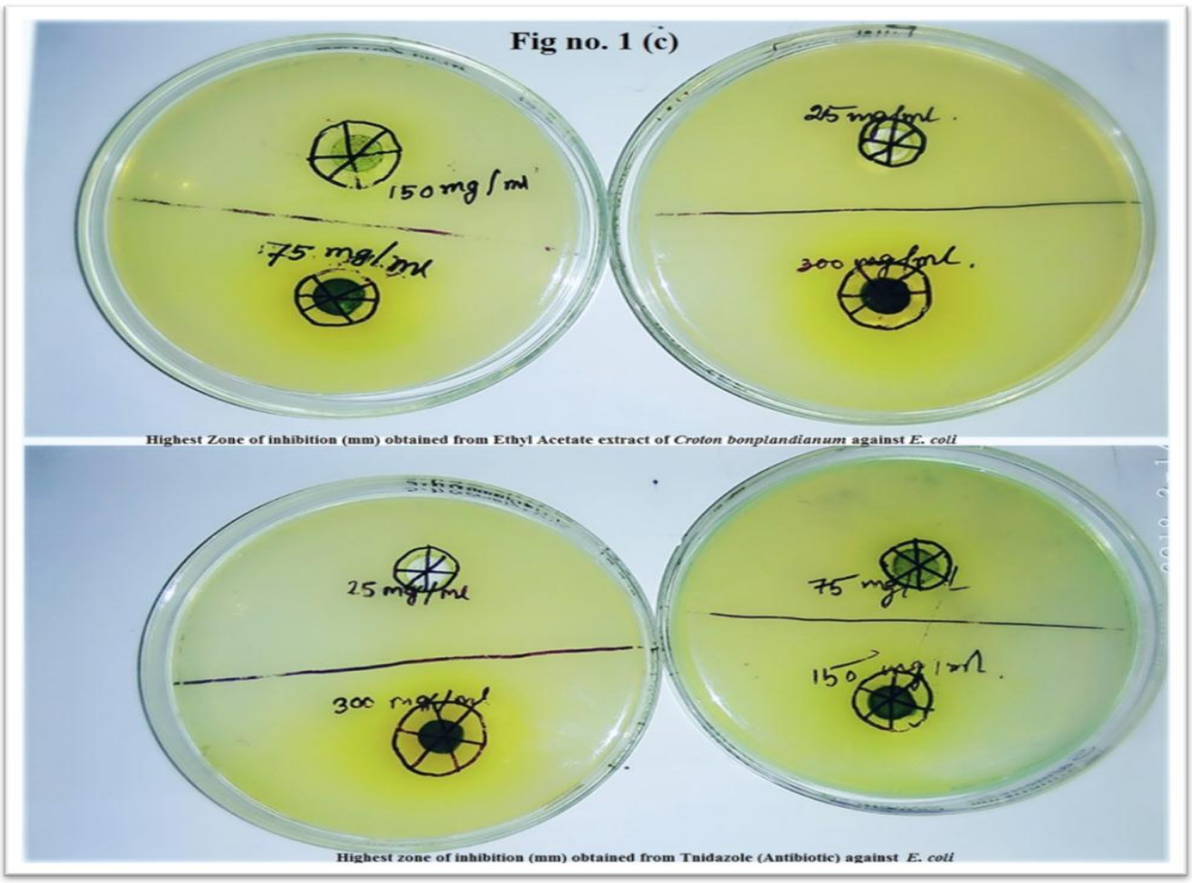
Treatment with Various ***extract of *Croton bonplandiaum*** against UTI causing pathogen- *Escherichia coli*

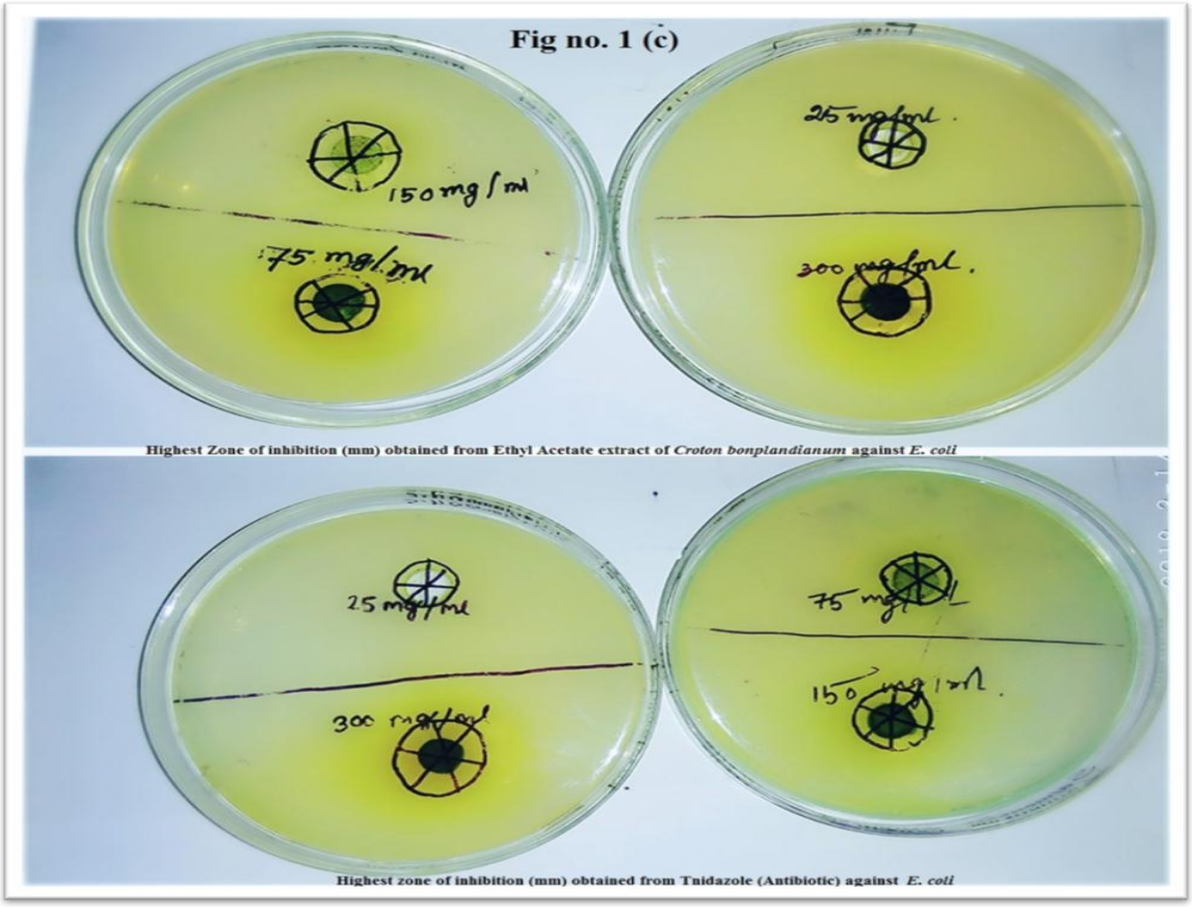
fig .- 1(A)

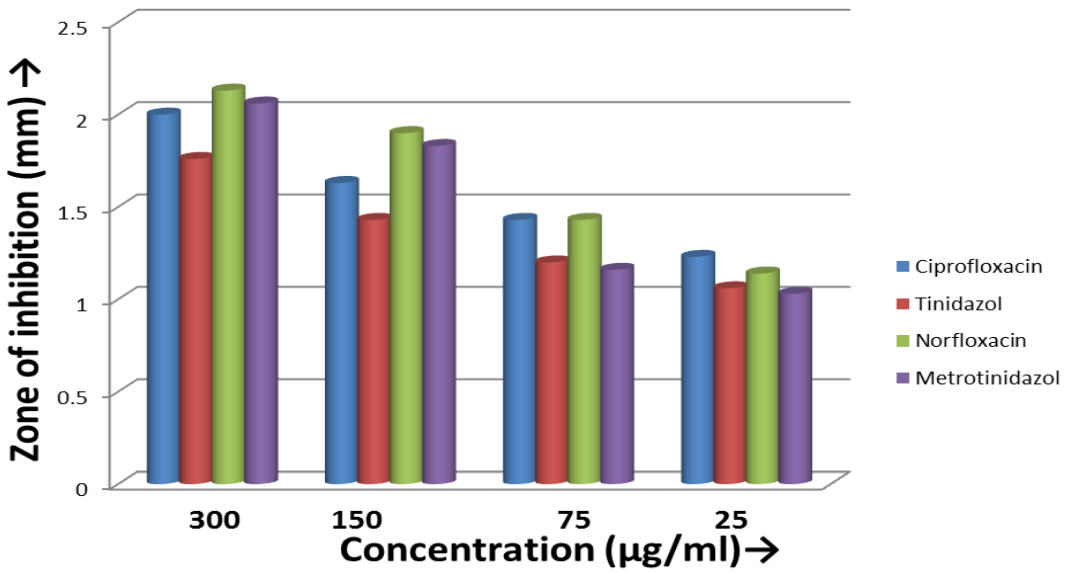


Treatment with Various **Antibiotics** against UTI causing pathogen- *Escherichia coli*

fig .- 1(B)

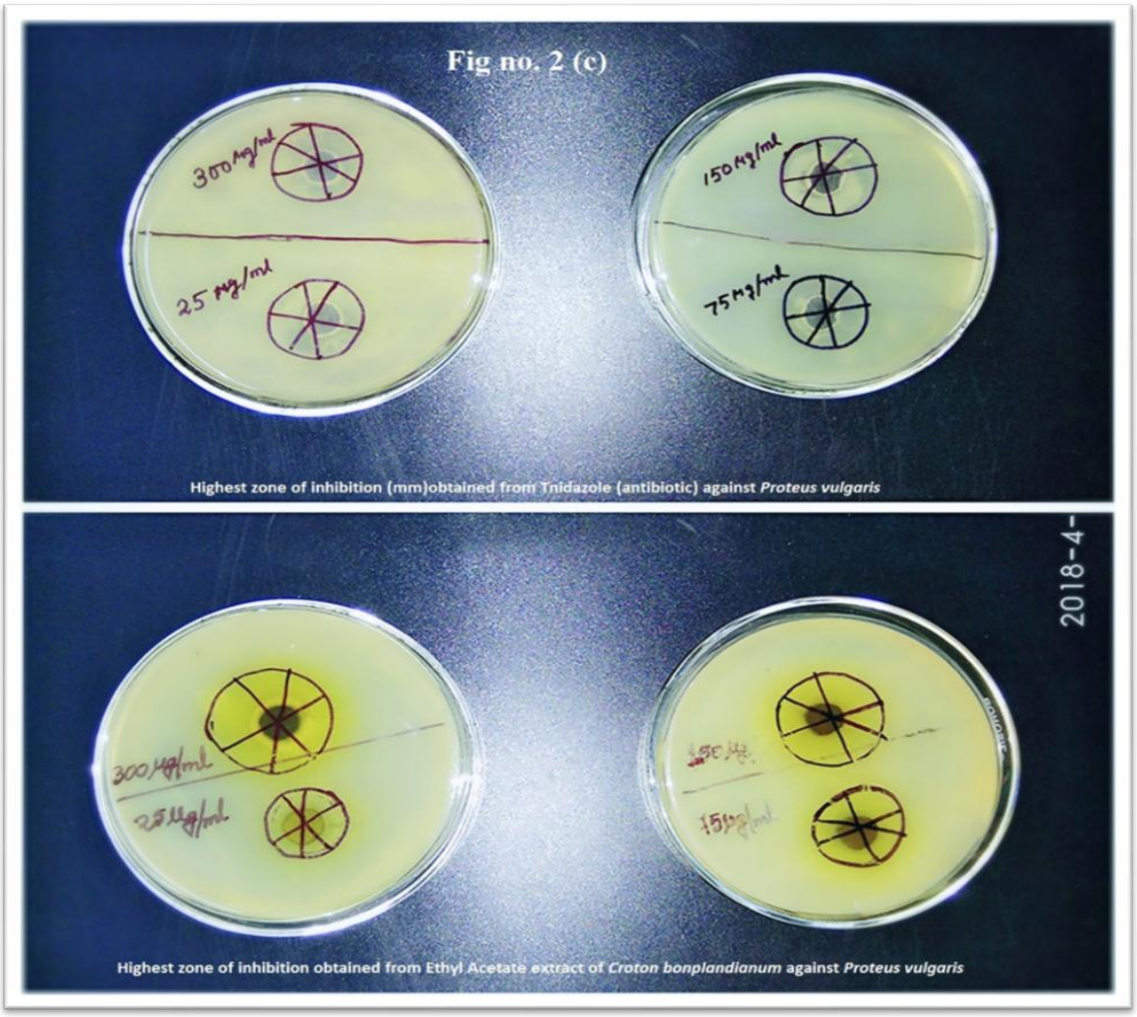


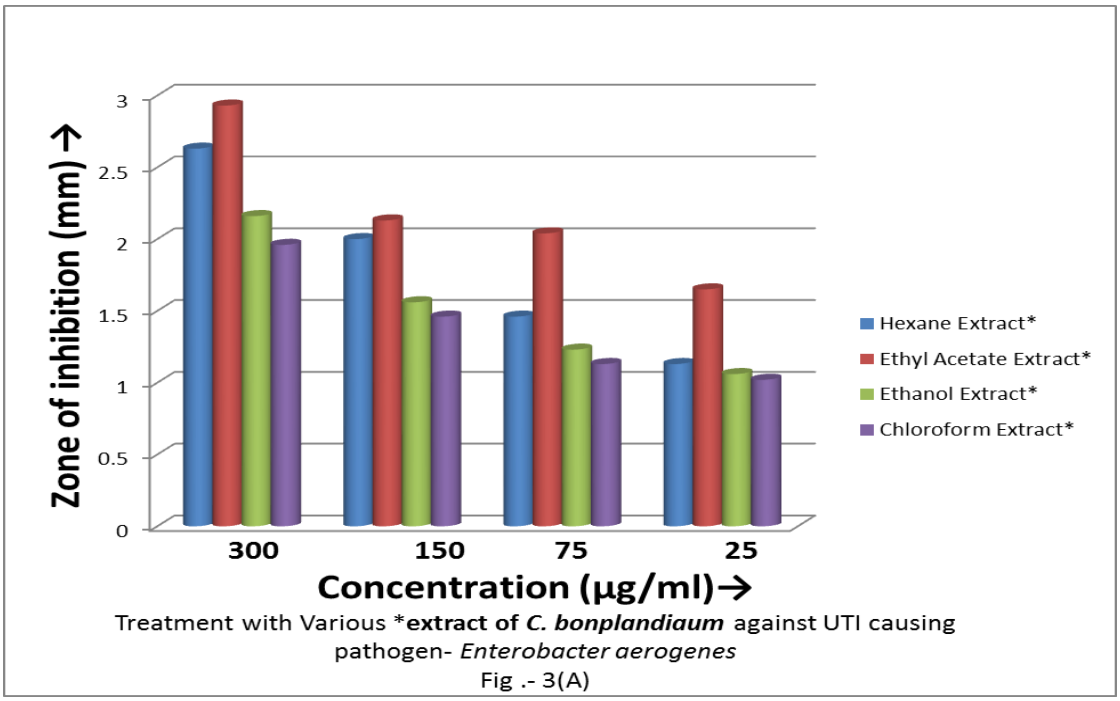




Treatment with Various **Antibiotics** against UTI causing pathogen- *Proteus vulgaris*

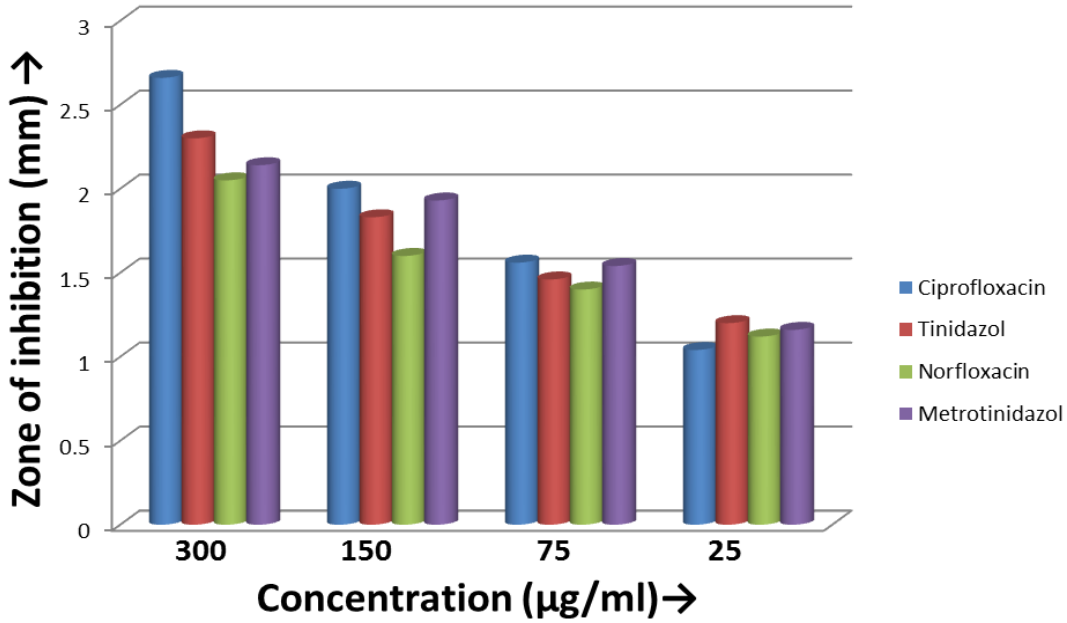
Fig .- 2(B)





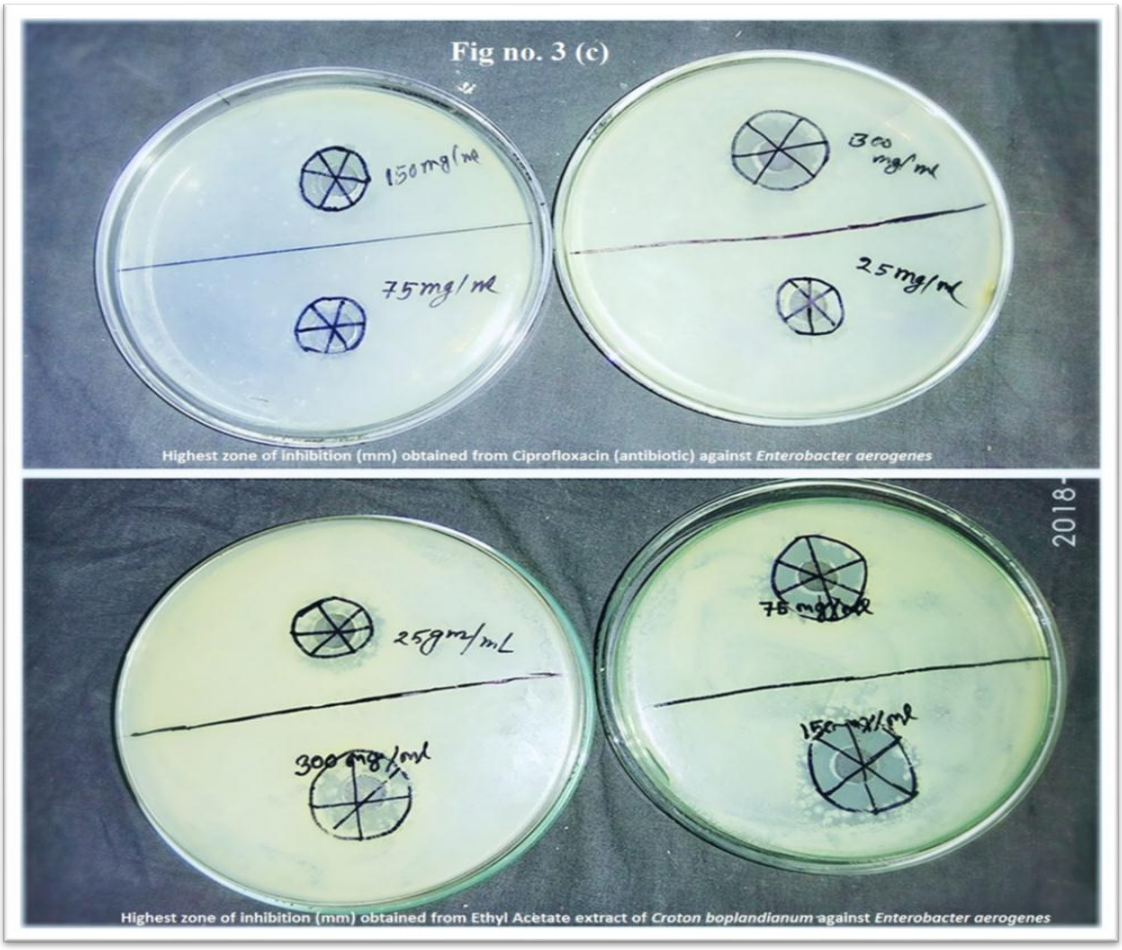
Treatment with Various ***extract of *C. bonplandiaum*** against UTI causing pathogen- *Enterobacter aerogenes*

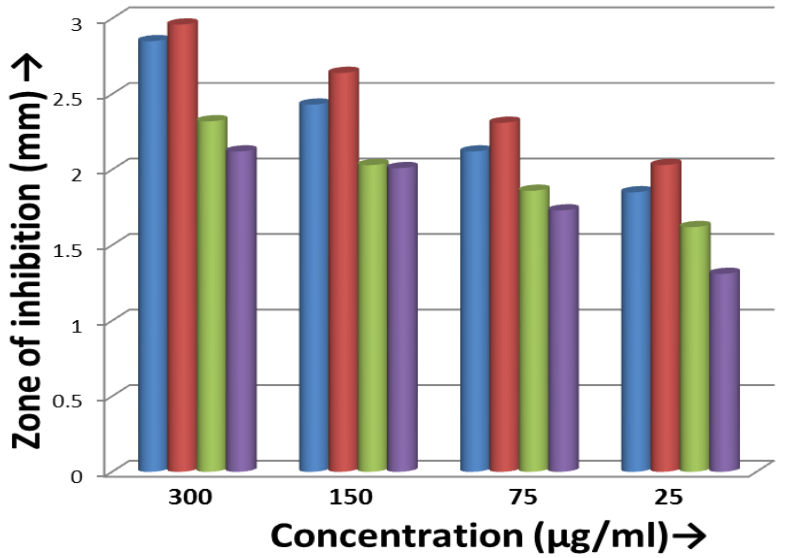
Fig .- 3(A)



Treatment with Various **Antibiotics** against UTI causing pathogen- *Enterobacter aerogenes*

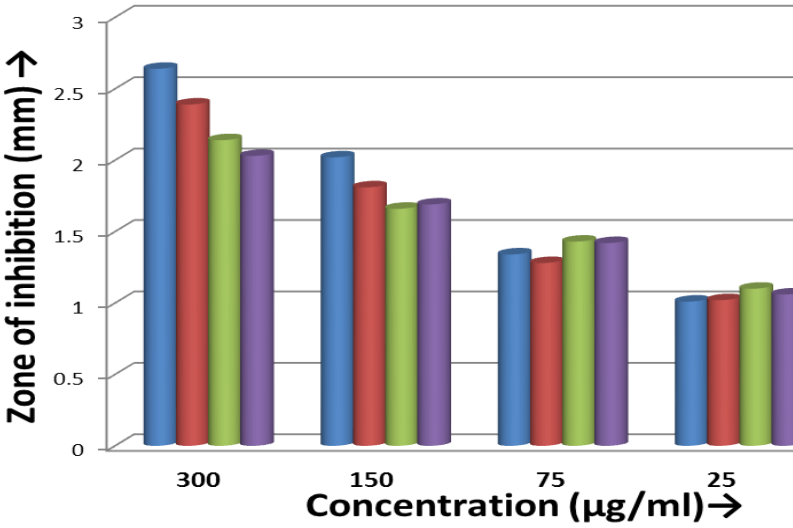
Fig.- 3 (B)





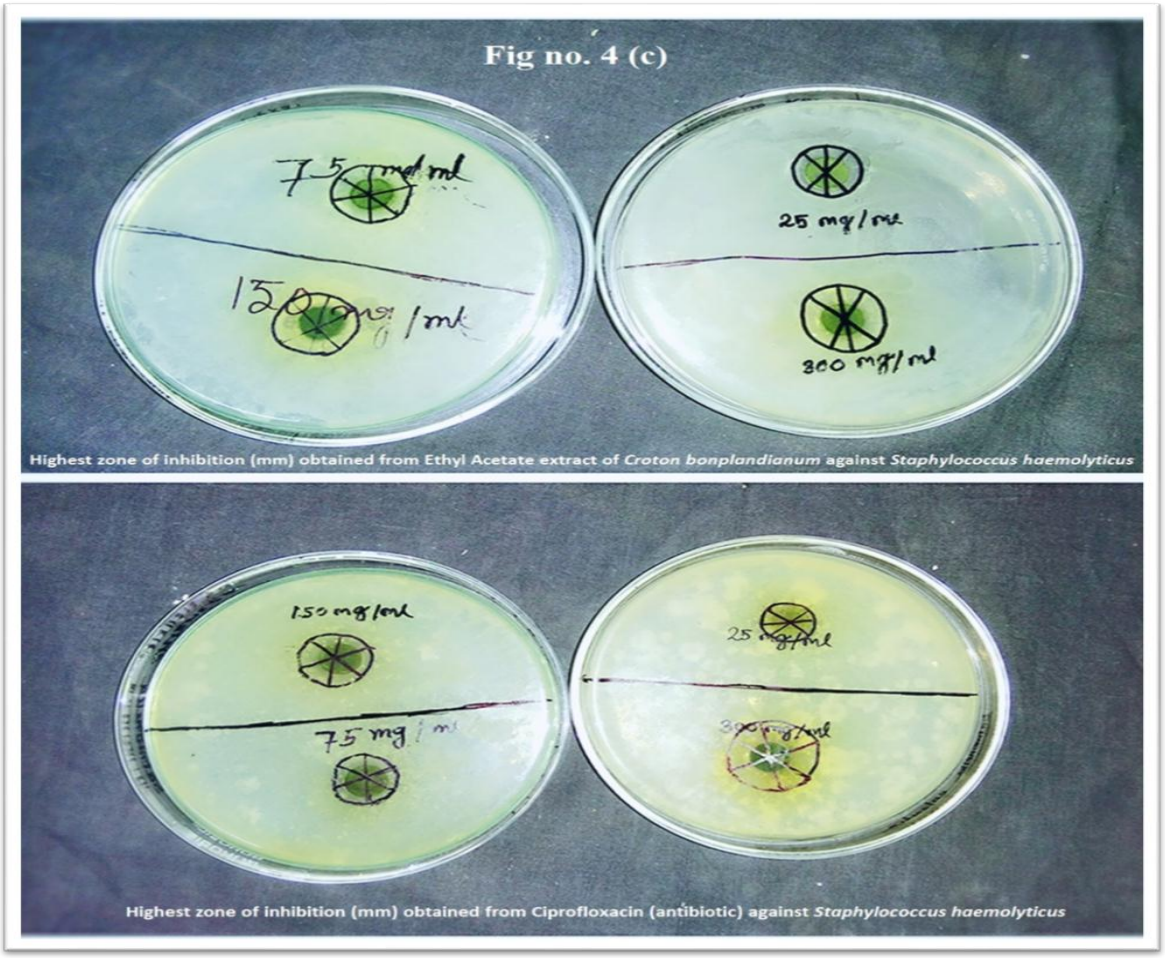
Treatment with Various ***extract of *C. bonplandiaum*** against UTI causing pathogen- *Staphyloccus haemolyticus*

Fig - 4 (A)



Treatment with Various **Antibiotics** against UTI causing pathogen- *Staphylococcus haemolyticus*

Fig.- 4 (B)



Cytotoxic Activity

In this study, Cytotoxicactivity of the Ethyl Acetate extract of leaves of *Croton bonplandianum* was screened against normal human erythrocytes. Cytotoxicactivity of the plant is expressed in percentage hemolysis and reported as mean±standard deviation of three replicates. All the samples exhibited very low haemolytic effect toward human erythrocytes. However, these extracts showed dose dependant increase in haemolytic activity (Figure 5).

Table no. 11: Cytotoxic Activity of Ethyl Acetate Extract of *Croton bonplandianum* against Human erythrocytes. [Data is represented as mean± standard deviation (n=0.6)].

SL No.	Concentration(µg/ml)	Dilution	Absorbence (O.D)	Cell viability (%)
1.	200	NEAT	0.86	62.5
2.	100	1:2	0.97	131.25
3.	50	1:4	1.02	162.50
4.	25	1:8	1.03	168.75
5.	Cell control	-	0.92	200

Four concentration are reported in Table 11. The Cytotoxicactivity of the different concentrations of Ethyl Acetate extracts was found in the following order: 200µg/ml< 100 µg/ml< 50 µg/ml< 25 µg/ml and we use Plot only Positive control of percentage of cell hemolysis in Figure no. 5.

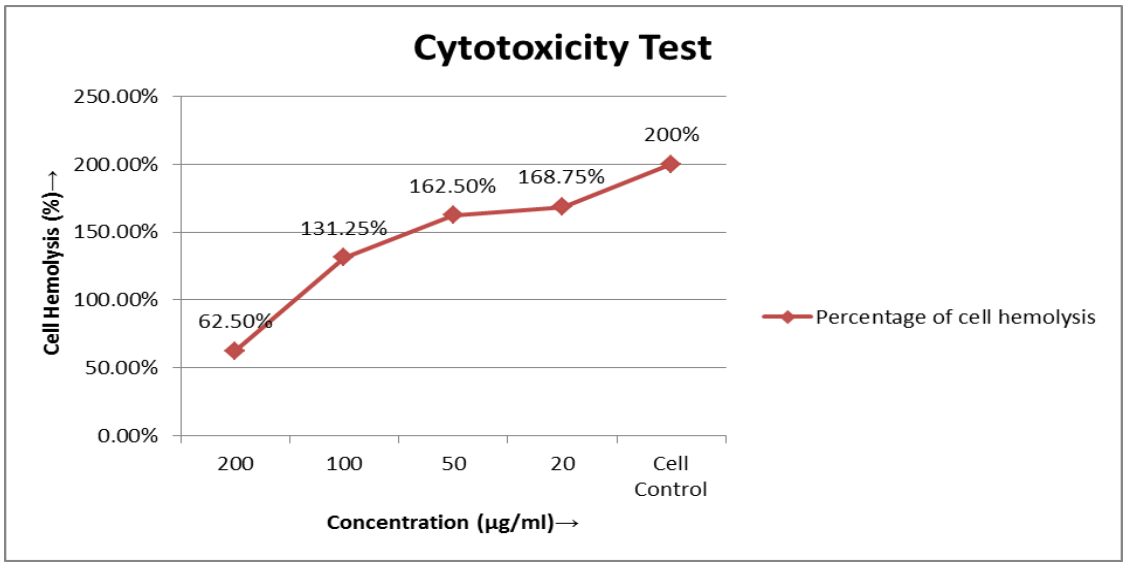


Fig. no. 5: Cytotoxicity test of Ethyl Acetate extract of *Croton bonplandianum*.

The results of this study concludes that the Ethyl Acetate extract from the leaves of *Croton bonplandianum* is non/less toxic to the human erythrocytes.

CONCLUSION AND DISCUSSION

Development of resistance by the microorganisms to chemotherapeutic agents appears to be a continuous process since the discovery of antibiotics. Scientists have realized an immense potential in natural products from medicinal plants to serve as an alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. From the above results we can conclude that the leaf extract of *Croton bonplandianum* has remarkable antimicrobial activity as compare to antibiotic activity. We know that organisms are gaining resistance day by day towards the multi drug or antibiotics, so that some natural product should be try to overcome these antibiotic resistant organisms. Moreover the plant extract of *Croton bonplandianum* have no Cytotoxic effect or Hemolytic activity as it shown above (Fig no. 5). That's why we can say that the Ethyl Acetate extract of *Croton bonplandianum* can be used for designing several drugs to treat complicated Urinary Tract Infections in future. Further work on isolation and characterization of active antimicrobial

compounds from medicinal plants and their pharmacodynamic study would be highly beneficial for the management of severe life threatening infections.

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