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Evaluation of the Phytochemical Investigation and Antimicrobial Efficacy of Different Extract of Leaves of *Croton bonplandianum* Against Some Pathogenic Bacteria *Salmonella enterica* ser. typhi and *Staphylococcus haemolyticus*

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ABSTRACT

Plants are healthy and natural resource of life. In particular, medicinal plants are of great importance with endless therapeutic properties useful for curing various diseases with an advantage of being natural. The present study is to evaluate the qualitative estimation of phytochemicals and antimicrobial activity of ethyl acetate, benzene, hexane, chloroform, methanol extracts of leaves of Croton bonplandianum against bacteria such as Staphylococcus haemolyticus : MTCC 3383 and Salmonella enterica ser. typhi: MTCC 8767. The process was carried out by agar well diffusion method. The extracts were poured into the wells at different concentrations like 25mg/ml, 50mg/ml, 150mg/ml and 300mg/ml. After incubation zones of inhibition were observed. As the concentrations of extracts increased the activity also increased and thus the zone of inhibition too increased. Among five extracts zone of inhibition was best in ethyl acetate extract. . In case of Salmonella enterica ser. typhi: (MTCC 8767) the ethyl acetate extract (300 mg/ml) showed maximum zone of inhibition 20.6±1mm, while chloroform extract (25mg/ml) showed minimum zone of inhibition 11.0±00. In case of Staphylococcus haemolyticus: (MTCC 3383) the ethyl acetate extract (300 mg/ml) showed maximum zone of inhibition 18.0±1mm, while benzene extract (25mg/ml) showed minimum zone of inhibition 11.0±2.0.Hence Croton bonplandianum can be used in developing drugs and medicines against various activities of bacteria. Study has also been shown the presence of various phytochemical constituents such as alkaloid, tannin, saponin thiamine, ascorbic acid, phenolic content in the leaf of Croton bonplandianum.

Key words: *Staphylococcus, Saponin thiamine, Ascorbic acid, Phenolic*

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INTRODUCTION

Croton bonplandianum, commonly known as three leaved caper (English), ban tulasi, jungle tulasi (Bengali), kalabhangre (Hindi), eliamanakkau (Tamil), kukka mirapa (Telugu), alpabedhi soppu (Kannada). This plant is about 60 cm high perineal herbs and can be found in waste lands and road side areas. Following and fruiting time of this plant is September to December [1]. The exotic weed, *C. bonplandianum* (Euphorbiaceae) generally distributed in the wastelands of tropical and subtropical regions of Madhya Pradesh, India. *Croton bonplandianum* Bail. is a monoecious exotic weed. We call it as ban tulsi because resemblance of leaves and flower to that of tulsi. It is an annual herb plant grows mainly as a bush, profoundly grows around the canal, river banks, big drainage, and waste lands etc areas. It has been reported that this plant is native to Southern Bolivia, Paraguay, South Western Brazil, North Argentina, Bangladesh, South America, India and Pakistan. In India it is widely distributed in the Sub-Himalayan region of West Bengal. Leaf is alternatively arranged around the steam, lance shaped with toothed margins. Normally the crotons are famous for its lovely foliage. At some regions this weed has already identified as noxious .Touching eyes after touching its leaves and flowers causes inflammation. Small white flowers borne are long racemes at the end of branches. Flowers have 5 petals and 5 sepals. Flowers are shortly stalked. It's fruit is oblong capsule like. It has warty surface. Knowledge on plants by the Indigenous people provides an insight to the researchers to screen traditionally used plants for active principles against various human pathogens. Modern knowledge on medicinal plant research till contains at least 25% drugs and many others, which are synthetic analogues, built on prototype compounds isolated from medicinal plants. *C. bonplandianum* possess immense medicinal value and its stem latex is used by different region as a medicinal plant for the treatment of fresh cuts and wounds to stop bleeding. It has got antimicrobial activity and act as good medicine for skin diseases, cut and wounds and also claimed to have the antiseptic properties. Diterpene resins present in *C. bonplandianum* stem is also used experimentally for cancer therapy and conceivably result was achieved and its methanolic extract possess tremendous importance for its antitumor potentiality which was evaluated with phytotoxic analysis[2]. Local people use the root as well as stem of *C. bonplandianum* against snake bite in the remote areas of West Bengal, India. The parts of plant are widely used in traditional system of medicine such as hepatoprotective, swelling of the body, cure against ring worms and skin disease, antihypertensive, antioxidant, wound healing, antifungal, antimicrobial, antidiabetic, antitumor, anticancer, acute constipation, abdominal dropsy, internal abscesses, antifertility, antispasmodic, antiseptic, antidote, analgesic, repellent property against insects, nematicide, anticoronary, anti-inflammatory, larvicidal activity, antihelmentic, this is also used for treatment of cholera, boils, bowel complaints, chicken pox, diarrhoea, dysentry, eye diseases, cold and coughs, epilepsy, gastric disorders, insanity, jaundice, liver complaints, scurvy, sprains, malaria, rheumatism, and so on. The fresh juice of the plant is used against headache. Due to its slow rate of conventional multiplication, the plant is very high in demand. In this review report we collected information related to taxonomy, monographs, distribution, morphology, phytochemistry, traditional uses and pharmacological studies of *Croton bonplandianum* Baill plant in details. There are many contradictory theories on the subject of herbal medicines and their relationship regarding with human physiology and mental function[3,4]. There is a need to develop evaluative data by using sophisticated modern techniques of standardization of Ayurvedic formulations to tackle the issues of negative criticism of Ayurvedic formulations and increased toxicity reports[5]. *Croton bonplandianum* is also important its antioxidant and wound healing property[6]. It has also the repellent property against the insects[7]. The fresh juice of the plant is used

against headache byethnic groups. Latex of plants has healing effect on wounds and cuts[8,9]. The present paper deals with the investigation of different types of phytochemicals such as tannin, phlobatannin, terpenoid, glycoside, phenolic, flavonoid, steroid, anthraquinone, saponin, alkaloid, cholesterol, carbohydrate and protein for a clear understanding regarding the phytochemical status of the stem of *C. bonplandianum*. These kinds of phytochemical investigation will help in understanding the phytochemical composition and safety of herbal formulation. This present study also deals with the antibacterial activity of leaf extract of *Croton bonplandianum* against Gram positive (*Staphylococcus haemolyticus*: MTCC 3383) and Gram negative (*Salmonella enterica* ser. typhi: MTCC 8767) bacteria. *Salmonella enterica* (MTCC 8767) subsp. enteric serover Typhimurium is a leading cause of food borne salmonellosis in many countries. The number of antibiotic resistant isolates identified in humans is steadily increasing, suggesting that the spread of antibiotic resistant strains is a major threat to public health. It causes typhoid fever, enteric fever, gastroenteritis etc. *Staphylococcus haemolyticus*(MTCC 3383) is also a remarkable bacterial pathogen that is well known for its highly antibiotic resistant properties. The bacteria can cause a lot of human disease including prosthetic join infections or bactermia meningitis, skin or soft tissue infections.

MATERIAL AND METHOD

Selection and Collection of plant:

The plant *Croton bonplandianum* was selected for study of antibacterial activity and phytochemical analysis. The plant

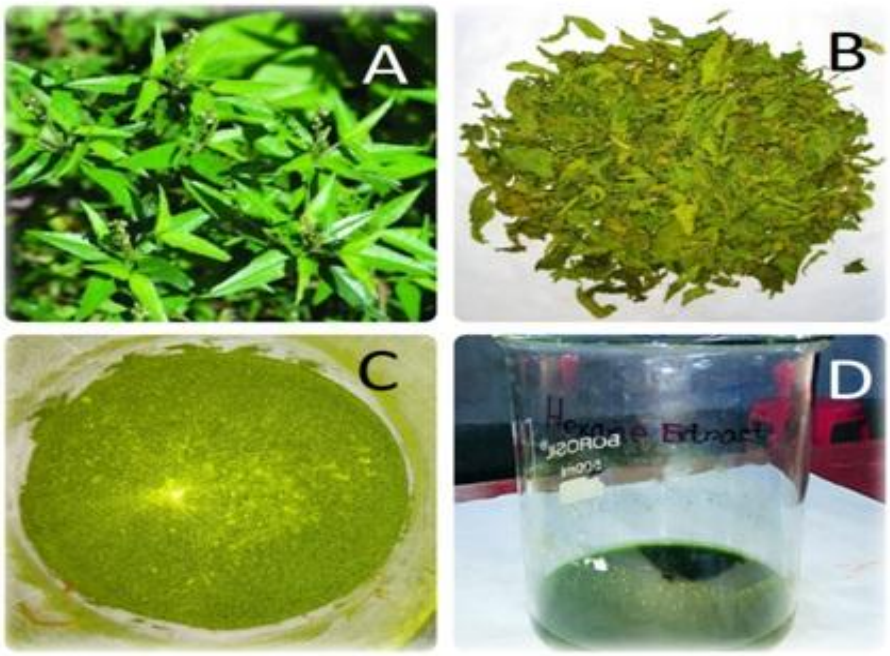


Figure 1: Extract preparation;

(A) *Croton bonplandianum* plant; (B) leaves separated and dried;

(C) leaves ground to powder; (D) extract obtained

Croton bonplandianum was collected randomly from the garden of Rabindra Mahavidyalaya, Champadanga, Hooghly, West Bengal, India. First leaves were washed with cold water and then without water. Then leaves were dried at room temperature (30-40 ^{0}C) for 7-10 days. Then leaves were air dried and then crushed to fine powder by mixer grinder and store in air-tight bottles (figure 1).

Preparation of different plant extract:

Benzene extract:

5gm of air-dried powder of leaves was mixed with 25ml of benzene in a conical flask and then kept on a rotary shaker for 10mints. Then they were bind with tissue paper and rubber band. Some holes were made so that air can pass through it and then take room temperature for 3-5 days for evaporate.

Hexane extract:

5gm of air-dried powder of leaves was mixed with 25ml of hexane in a conical flask and then kept on a rotary shaker for 10mints. Then they were bind with tissue paper and rubber band. Some holes were made so that air can pass through it and then take room temperature for 3-5 days for evaporate.

Chloroform extract:

5gm of air-dried powder of leaves was mixed with 25ml of chloroform in a conical flask and then kept on a rotary shaker for 10mints. Then they were bind with tissue paper and rubber band. Some holes were made so that air can pass through it and then take room temperature for 3-5 days for evaporate.

Ethyl acetate extract:

5gm of air-dried powder of leaves was mixed with 25ml of ethyl acetate in a conical flask and then kept on a rotary shaker for 10mints. Then they were bind with tissue paper and rubber band. Some holes were made so that air can pass through it and then take room temperature for 3-5 days for evaporate.

Methanol extract:

5gm of air-dried powder of leaves was mixed with 25ml of methanol in a conical flask and then kept on a rotary shaker for 10mints. Then they were bind with tissue paper and rubber band. Some holes were made so that air can pass through it and then take room temperature for 3-5 days for evaporate.

Preparation of extract concentration:

Four concentrations (25mg/ml, 75mg/ml, 150mg/ml and 300mg/ml) were made from each of the six extracts (Benzene, Chloroform, Hexane, aqueous, Ethyl acetate, Methanol extract). In every case 3 gm of Extract was mixed with 10ml DMSO (dimethyl sulfoxide) to prepare 300 mg/ml stock concentration. Then other three concentrations were made by adding extra DMSO with the Stock in other test tube.

Collection of Microorganisms:

The pathogenic strain of *Salmonella enterica* ser. typhi: MTCC (8767) and *Sthphylococcus haemolyticus* were used in the present study. These strains of bacteria were purchased from Microbial Type Culture Collection (MTCC), Institute of Microbial technology (IM-TECH), Chandigarh, India. The bacterial strains were maintained in Muller Hinton Agar (MHA, pH-7.2) at 37^{o}C .The stock culture slants were maintain at 4^{o}C.

Microbiological assay:

Agar disc diffusion method:

The antibacterial screening of leaf extract of *C. bonplandianum* was prepared by dissolving 3gm of each extract separately in 10ml Dimethyl Sulphoxide (DMSO). From this 25mg/ml, 75mg/ml, 150mg/ml, 300mg/ml concentration were taken for the analysis of antibacterial activity. A hollow tube was heated and pressed above the inoculated agar plate. It was removed immediately by making a well in the plate; two wells on each plate were made one each for DMSO control.

Medium:

3.8 g of Mueller Hinton Agar (MHA) was added to 100ml of distilled water and autoclaved at 121^{0}c for 15 minutes at 15 lbs and poured in sterile petri plates up to a uniform thickness of approximately 4mm and the agar is allowed to set at ambient temperature and used.

Inoculums and incubation:

0.1mg of bacterial cultures was transferred to the agar plates. The inoculated plates were allowed to stand for 5 min, before making wells for different concentrations to be tested. The extracts of leaf of *C. bonplandianum* were loaded at different concentrations in the well on agar plate. Then bacterial cultures and incubated at 37ºC for 24-48 hours in an incubator.

METHODS

After incubation the diameter of zone of inhibition around the well was measured using zone reader[10]. Corresponding 3 values of zones

of inhibition for each concentration of *C. bonplandianum* extract were taken. The values so obtained were compared within the group (same concentration of extract) and with different groups (different concentrations of extract) for different bacteria and statistical analysis was done.

RESULTS AND DISCUSSION

The result of the present study showed significant antibacterial activity against *Staphylococcus haemolyticus*: (MTCC 3383) and *Salmonella enterica* ser. typhi: MTCC 8767. Crude extracts obtained from leaf of *C. bonplandianum* using different solvents like benzene, methanol, ethyl acetate, chloroform and hexane were screened for antibacterial activity against bacterial pathogens. In present investigation all the extracts of *C. Bonplandianum* used for the study showed antimicrobial activity against Gram positive and Gram negative bacteria. Antibacterial activity was tested on Muller Hinton Ager (MHA) plates by agar cup method. Various concentrations of extracts were prepared.

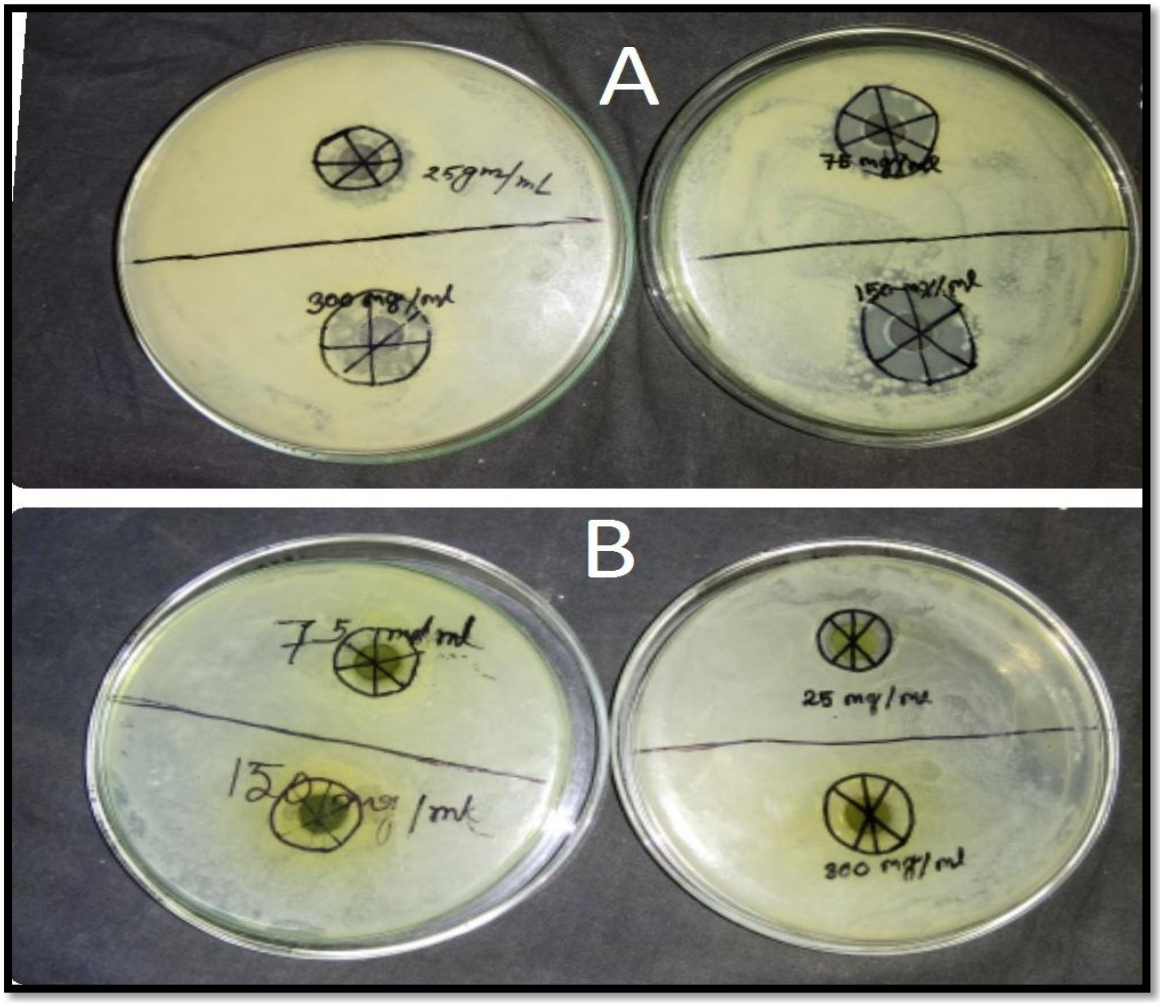


Fig. 2: (A) Antibacterial activity of ethyl acetate extract on *Salmonella enterica* ser. typhi: MTCC 8767
(B) Antibacterial activity of benzene extract on *Salmonella enterica* ser. typhi: MTCC 8767

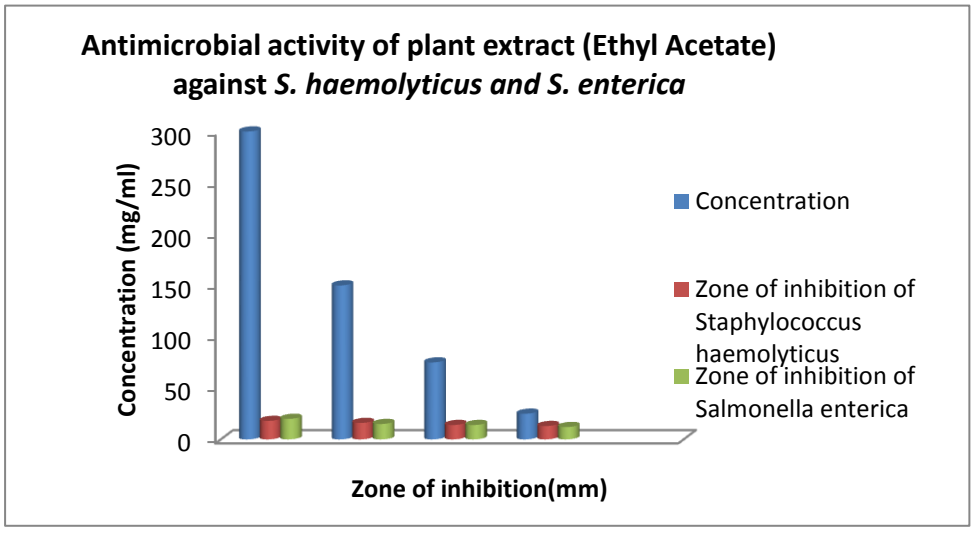
The whole process was tested against two species of bacteria; Gram positive *Staphylococcus haemolyticus*: (MTCC 3383) and Gram negative *Salmonella enterica* ser. typhi: (MTCC 8767) were taken for study. There was no zone of inhibition in the negative control by DMSO. The antibacterial efficacy of various solvent extracts of *C. bonplandianum* showed varied level of inhibition against the bacteria (table 1). The antibacterial activity of the various solvent extracts of leaf of *C. bonplandianum* against bacterial isolates showed best results at the concentration of 300 mg/ml and the results are

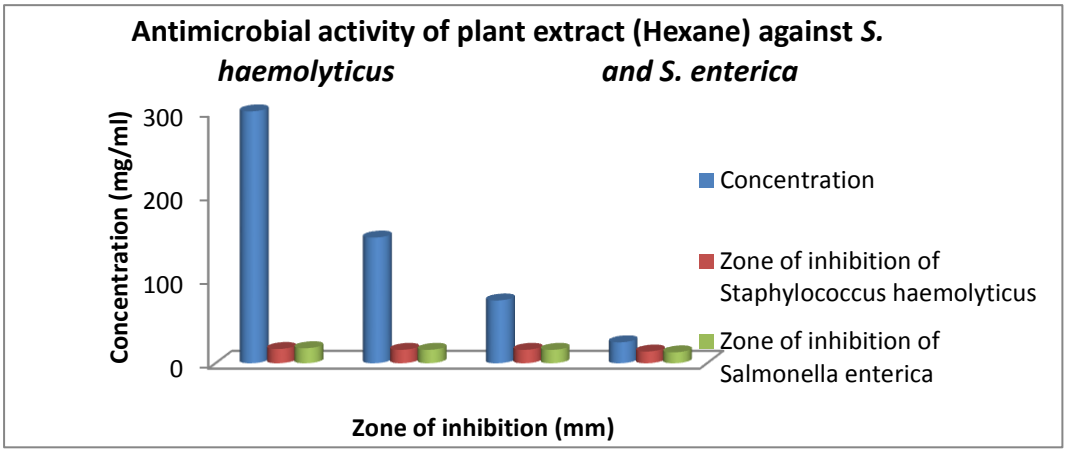
shown in figure 2. *C. bonplandianum* leaves extract showed increasing zones of inhibition with increasing concentration against all bacteria. Mean zone of inhibition and standard deviation for each concentration and each bacterium was calculated for analysis. In case of *Salmonella enterica* ser. typhi: (MTCC 8767) the ethyl acetate extract (300 mg/ml) showed maximum zone of inhibition 20.6±1mm, while chloroform extract (25mg/ml) showed minimum zone of inhibition 11.0±00. In case of *Staphylococcus haemolyticus*: (MTCC 3383) the ethyl acetate extract (300 mg/ml) showed maximum zone of inhibition 18.0±1mm, while benzene extract (25mg/ml) showed minimum zone of inhibition 11.0±2.0.

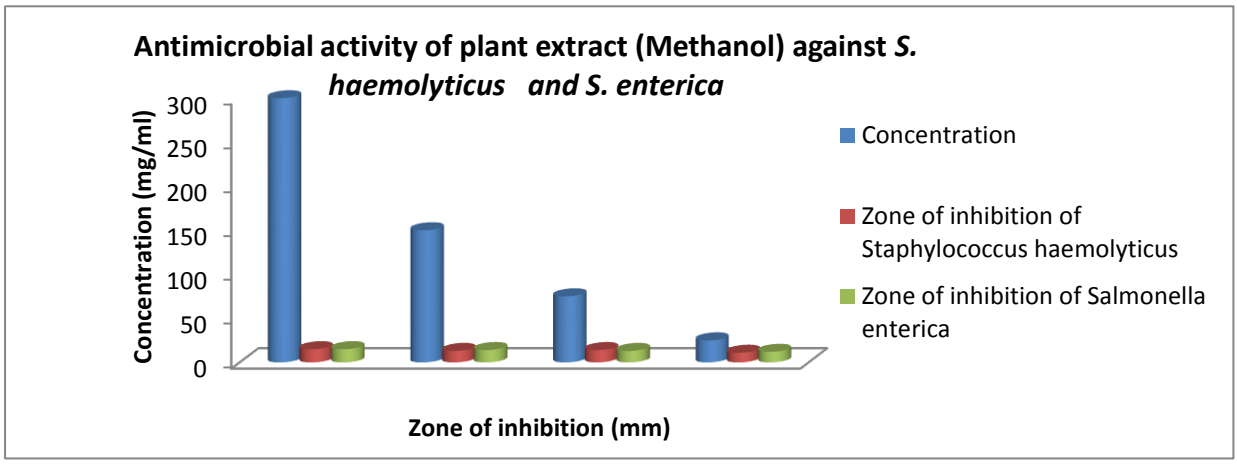
Table 1: Antibacterial activity of different solvent extracts of leaf of *C. Bonplandianum*

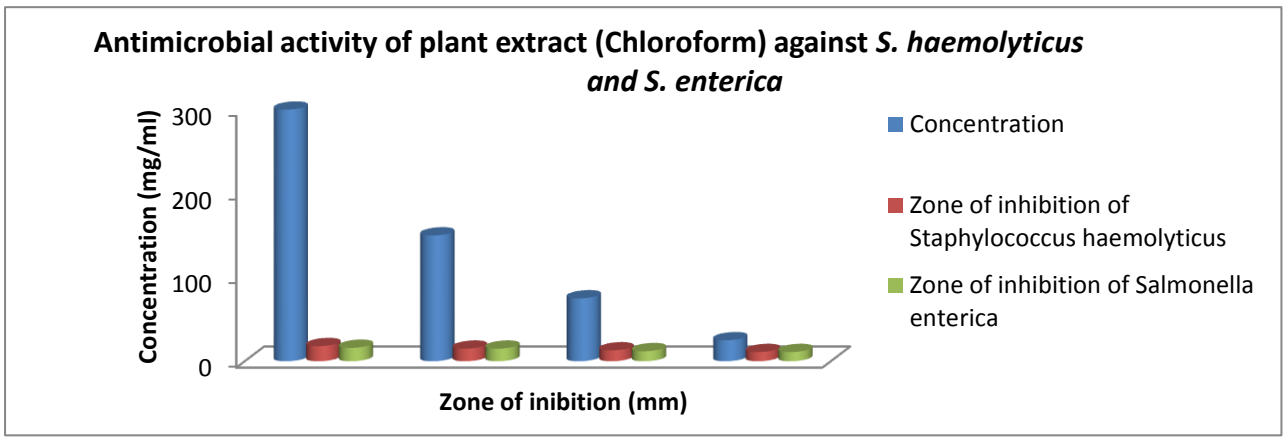
Name of the solvent extracts	Concentration of the extract	Diameter of zone of inhibition (mm)	
		Name of the bacterial species	
		Staphylococcus haemolyticus	***Salmonella enterica* ser. typhi**
Ethyl acetate	300 mg/ml	18.0±2.0	20.6±1.0
	150mg/ml	16.3±1.0	15.3±00
	75mg/ml	14.6±2.0	14.0±00
	25mg/ml	13.4±1.0	12.3±1.0
Hexane	300 mg/ml	17.6±1.0	18.6±1.0
	150mg/ml	16.7±1.0	16.7±1.0
	75mg/ml	16.0±00	16.0±2.0
	25mg/ml	14.6±1.0	13.6±1.0
Chloroform	300 mg/ml	18.0±3.0	16.0±1.0
	150mg/ml	15.0±1.0	15.0±00
	75mg/ml	13.0±1.0	12.0±1.0
	25mg/ml	11.3±1.0	11.0±00
Methanol	300 mg/ml	15.6±1.0	15.6±1.0
	150mg/ml	13.0±00	14.3±1.0
	75mg/ml	14.3±1.0	13.0±2.0
	25mg/ml	11.6±1.0	12.0±00
Benzene	300 mg/ml	15.3±1.0	14.6±1.0
	150mg/ml	14.0±00	15.0±00
	75mg/ml	14.3±1.0	13.3±1.0
	25mg/ml	11.0±2.0	12.0±00

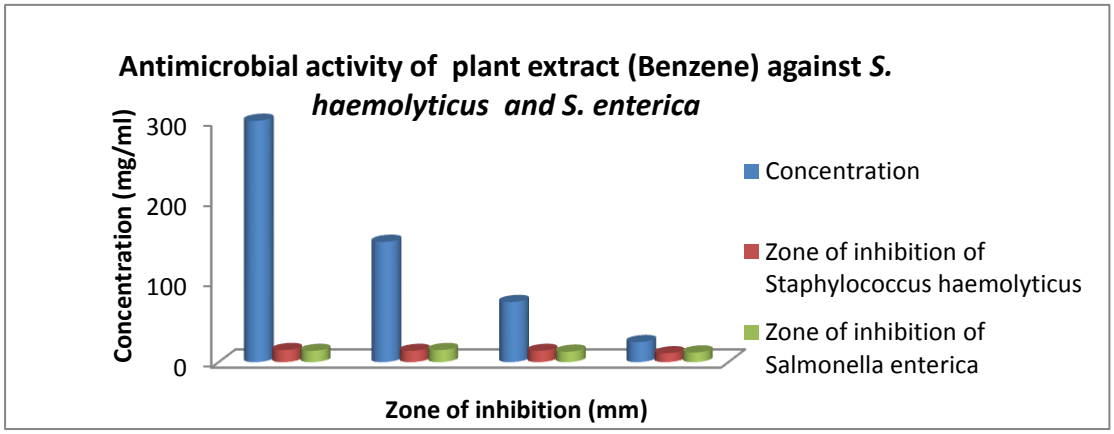
Values are expressed as mean ± standard deviation.




Antimicrobial activity of plant extract (Hexane) against S. haemolyticus and S. enterica
Concentration (mg/ml)
300
200
100
0
Concentration
Zone of inhibition of Staphylococcus haemolyticus
Zone of inhibition of Salmonella enterica
Zone of inhibition (mm)


Antimicrobial activity of plant extract (Methanol) against S. haemolyticus and S. enterica
Concentration (mg/ml)
300
250
200
150
100
50
0
Concentration
Zone of inhibition of Staphylococcus haemolyticus
Zone of inhibition of Salmonella enterica
Zone of inhibition (mm)


Antimicrobial activity of plant extract (Chloroform) against S. haemolyticus and S. enterica
Concentration (mg/ml)
300
200
100
0
Concentration
Zone of inhibition of Staphylococcus haemolyticus
Zone of inhibition of Salmonella enterica
Zone of inibition (mm)



Phytochemical estimation:

Extract preparation:

20 gm of air-dried powder was taken in 100 ml of each solvent (methanol, ethyl acetate, hexane, benzene, chloroform) in a conical flask, plugged with cotton wool and then kept on a rotary shaker. After 24 hours the supernatant was collected and the solvent was evaporated.

Phytochemical studies:

The methods described by Harborne were used to test for the presence of the active ingredients in the test sample[11].

Test for steroids:

A 10 ml of plant extract (methanol, ethyl acetate, hexane, benzene, chloroform) was evaporated to a dry mass and the mass is dissolved in 0.5 ml of chloroform. Acetic anhydride [0.5 ml] and 2 ml of concentrated sulphuric acid were added to above[12].

Test for alkaloids:

The plant extract (methanol, ethyl acetate, hexane, benzene, chloroform) [0.5 g] was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100ml with distilled water[12,13].

Test for tannins:

About 1 g of plant extract powder was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl3 were then added[14].

Test for flavonoids:

A few drops of 1% NH_3 solution is added to the plant extract [0.5 g] in a tube for observation of Yellow coloration[12].

Test for reducing sugar:

To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution were added at hot and observed for brick red precipitate[15].

Table 1: Display the presence/ absence of different phytochemicals in the leaf of *C. bonplandianum*

Phytochemicals	Ethyl acetate	Hexane	Chloroform	Methanol	Benzene
Tannin	+	+	-	+	-
Alkaloid	+	+	+	-	+
Flavonoid	+	-	+	+	+
Phenolic	-	+	-	+	-
Steroid	+	+	+	+	+
Saponin	+	-	+	+	+
Reducing sugar	-	+	+	+	+

CONCLUSION

Traditional medicinal practice has been known for centuries in many parts of the world for the treatment of various diseases [16]. The use of antibiotics has revolutionized the treatment of various enteric bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganism, thus necessitating the need for development of novel antimicrobials. The present study may conclude that the leaves of *Corton bonplandianum* possess various phytochemicals like alkaloid, saponin, flavonoid, protein and tannin in a high quality and possess various bioactive properties. Through the world *Corton bonpladianum* is well recognized in different pharmacological practices and the presence of high quantities of those bioactive phytochemicals may attribute to its medicinal value. The bio-inductive study will be helpful for improving the yield of these metabolites. All these information leads us to conclude that leaves of *Corton bonpladianum* harbours immense qualities and can future prove a pivotal role in the field of phytochemical research and developing drugs and medicines.

Future aspect:

Scientific research and inventions have always been the thrust of mankind and is largely responsible for the standard of living he has today. Natural resources of a country are of primary importance for the economic development. Plants were in existence even before man came into existence. The importance of plants in the medical treatment cannot be overestimated. Hence, the global knowledge about Indian herbals will hopefully be enhanced by information on the evidence-base of these plants. The emerging field of herbal products industry holds a great potential to the economic development of the Indian region. There is an increasing trend of using plants as a source of food, medicine and perfumes. In conclusion, the results obtained in this study indicated that the traditional plant, *C. bonplandianum* generally used as potential source for useful drugs like antiinflammatory, anticancer, antimicrobial, insectifuge, nematicide, anticoronary, wound healing, hepatoprotective activities demonstrated broad spectrum antibacterial activity against bacterial isolates of both Gram negative and Gram-positive bacteria. So, further research is needed to isolate, identify, characterize and elucidate the structure of these bioactive compounds responsible for medicinal values of *C. bonplandianum*. In the present study various major phytocompounds were identified in leaves of *C. bonplandianum*. *Staphylococcus haemolyticus*: (MTCC 3383) is a coagulase-negative member of the genus Staphylococcus. The bacteria can be found on normal human skin flora and can be found in axillae, perineum, and ingunial areas of humans. *S.haemolyticus*: (MTCC 3383) is also the second most common coagulase-negative staphylococci presenting in human blood. However, recent studies indicate that coagulase-negative staphylococci have emerged as a major cause of opportunistic infection. *Staphylococcus haemolyticus*: (MTCC 3383) itself is also a remarkable opportunistic bacterial pathogen that is well-known for its highly antibiotic-resistant phenotype. The bacteria can cause various human diseases. The ability of the bacteria to simultaneously resist against multiple types of antibiotic has been observed and studied for a long time[17]. Common antibiotics that are subject to resistance in *S. haemolyticus* include methicillin, gentamycin, erythormycin, and uniquely among staphylococci, glycopeptide antibiotics. The resistance genes for each type of antibiotic can be located on the chromosome (methicillin), on the plasmids (erythromycin) or on both chromosome and plasmids [18]. We have to study the multi-drug resistant ability of *Staphylococcus haemolyticus* and its pathogenic characters. In present study it has been known that *Staphylococcus haemolyticus* which has resistant properties to many drugs such as methicillin, gentamycin, erythromycin but the leaves extract of *Croton. Bonplandianum* is able to suppress the growth of pathogenic *Staphylococcus haemolyticus*: (MTCC 3383). It can be concluded that in future, drugs might

be designed to inhibit the growth of *Staphylococcus haemolyticus*: (MTCC 3383).

REFERENCES

1. Reddy, K.R., Folk medicines from Chittor District Andra Pradesh, used in treatment of jaundice. Pharmaceutical Biol., **26:** 137-140 (1995)
2. Islam, M,S., Rahman, M.M., Rahman, M.A., Qayum, M.A., Alam, M.F., In vitro evaluation of *Croton bonplandianum* Baill. As potential antitumor properties using *Agrobacterium tumefaciens*. J Agr Technol, **6:** 79-86 (2010).
3. Sriwastava, N.K., Shreedhara, C.S., Aswatha, R.H.N.,Standardization of Ajmodadichurna, a polyherbal formulation. Pharmacognosy Res, **2:** 98-101 (2010).
4. Qureshi, S., Diab, A.A., Al-Anazi, F.A., Al-Hassan, M.I., Qureshi, M.F., Qureshi, V.F., *et al*. Negative aspects of the beneficial herbs: An over view. J Herb Med Toxicol, **6:** 1-14 (2012).
5. Saper, B.R., Kales, N.S., Paquin, J., Burns, J.M., Eisenberg, M.D., Davis, B.R., *et al*. Heavy metal content of Ayurvedic herbal medicine products. Jama, **292(23):** 2868-2873 (2004).
6. Chopra, R.N., S.L., Nayyar, and I.C., Chopra, Glossary of Indian medicinal plants. National Glossary of Indian medicinal plants. New Delhi. (1956).
7. Nishanta, R., Harris, C.S., Towers, G.H.N., Antimicrobial activity of plants collected from serpentine outcrops in SriLanka. Pharmaceutical Biology **40(3):** 235-244 (2002).
8. Das, A.J., B.K. Dutta, and G.D. Sharma., IndianTraditional Knowledge, **7(3):** 446-454 (2008).
9. Chandel, K.P.S., G. Shukla., and N. Sharma., Biodiversity in Medicinal and Aromatic Plants in India. National Bureau of Plant Genetic Resources, New Delhi. (1996).
10. Vennila, and Udayakumar; BMRJ, 5(1): 83-93, Article no. BMRJ.2015.010, British Microbiology Research Journal **5(1):** 83-93, (2015).
11. JB Harborne; H Baxter. Phytochemical Dictionary, Taylor and Francis Washington DC, 1765. (1993).
12. A.A. Siddiqui; M Ali; Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, 126-131 (1997).
13. W.C. Evans; GETrease. Tratado de farmacognosia, 12th ed. Nueva….. Federal University of Paraná, Caixa Postal 5336, CEP 80040-980 (1988).
14. M.A. Iyengar., Study of Crude Drugs. *Manipal Power Press, Manipal, India*, 8, 2 (1995).
15. S. Sadasivam; B. Theymoli., *Practical Manual* [*Undergraduate*], Tamil Nadu Agricultural University, Coimbatore, 2 (1985).
16. Dr. G. Indira priyadarsini, Dr. V. Sowbhagya rani . Traditional Medicinal Knowledge in India- An Appraisal. Socio-Economic voices. report. dec 2010-jan (2011).
17. Falcone, M., et al. Staphylococcus haemolyticus endocarditis: clinical and microbiologic analysis of 4 cases. Diagn. Microbiol. Infect. Dis. 57, 325-331 (2007).
18. Froggatt, J. W., Johnston, J. L., Galetto, D. W. & Archer, G. L. Antimicrobial resistance in nosocomial isolates of Staphylococcus haemolyticus. Antimicrob. Agents Chemother. 33, 460-466 (1989).