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A Review on Traditional and Pharmacological Uses of *Croton bonplandianum* with Special Reference to Phytochemical Aspect

Tanmay Ghosh[1], M. K. Biswas[2*], Pradipta Roy[1] and Chiranjib Guin[1]

[1]Department of Microbiology, Rabindra Mahavidyalaya, Champadanga, Hooghly, W.B., India.
[2]Department of Plant Protection, Palli Siksha Bhavana, Visva-Bharati, Sriniketan, Birbhum, W.B., India.

Authors' contributions

This work was carried out by the team of four authors. Author TG was the main architect who designed the study, provided valuable information to others, suggested about the source of secondary data and finally prepared the manuscript. Author MKB finally checked the manuscript. Authors PR and CG collected the information from various literature and sources, analyses the study. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Plant-based traditional medicine system continues to play a vital role in the healthcare system with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. 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. The ongoing growing recognition of medicinal plants is due to escalating faith in herbal medicine. There are many contradictory theories on the subject of herbal Medicines and their relationship regarding with human physiology and mental function. There is a need to develop

**Corresponding author: E-mail: mohankumar.biswas@visva-bharati.ac.in;*

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. These kinds of phytochemical investigation both qualitatively and quantitatively will help in understanding the phytochemical composition and safety of herbal formulation. *Croton bonplandianum* Baill is often called Ban Tulsi (Jungle tulsi). 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. 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.

Keywords: Pharmacological uses; Croton bonplandianum; phytochemical; herbal medicine; ayurvedic formulation; biological activity; bioactive compounds.

1. INTRODUCTION

Croton bonplandianus Baill. is a monoecious exotic weed. The plant is usually 30-40 cm in height, with whorled ranches which grows in sandy clay soil along road side, railway abandoned field in wide open ravines, and paddy or sugarcane fields. 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. Due to the resemblance of the leaves and flower cymes to that of Tulsi, this plant is often called Ban Tulsi locally in the Sub-Himalayan region of West Bengal. *C. bonplandianus* 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. There are many contradictory theories on the subject of herbal medicines and their relationship regarding with human physiology and mental function [1,2]. 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 [3]. These kinds of phytochemical investigation both qualitatively and quantitatively will help in understanding the phytochemical composition and safety of herbal formulation. *Croton bonplandianum* was selected to investigate its antioxidant and wound healing property [4]. *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 foundin waste lands and road side areas. Following and fruiting time of this plant is September to December [5]. Hence, the present study was aimed to determine the larvicidal effect of methanolic extracts of *C. bonplandianum* leaves against larvae of *A. aegypti.* The control mortality was corrected by Abbott's formula (1925). LC50 and LC 90 regressions and 95% confidence limit were calculated by using pro-bit analysis [6]. The exotic weed, *C. bonplandianum* (Euphorbiaceae) generally distributed in the wastelands of tropical and subtropical regions of Madhya Pradesh, India are reported to have many medicinal uses including the repellent property against the insects [7]. The seed of *Croton bonplandianum* contains diterpines, phorbolester, including 12-orthotrideconeoly-phorbol-13-acetate (TPA) and myristoyl phorbol acetate (MPA). TPA is a carcinogen, affecting prostaglandin metabolism [8,9]. The fresh juice of the plant is used against a headache by ethnic groups. The latex of plants has a healing effect on wounds and cuts [10,11]. the present paper deals with the investigation of different types of phytochemicals qualitatively and quantitatively 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. bonplandianus* which

may help future investigators in their Pharmacological analysis of this species. *Croton* species are used in a diverse way. Popular uses include treatment of cancer, constipation, diabetes, digestive problems, dysentery, external wounds, fever, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain, ulcers and weight-loss (Salatino et al., 2007). In some parts of India, peoples made a hot poultice of powdered leaves of *C. californicus* and use it as a pain reliever for rheumatism (Wilson et al., 1976). It has recently been shown in Kenya that *Croton* nuts, such as those from *C. megalocarpus*, are a more economical source of biofuel than Jatropha. In Kenya, Jatropha requires as much as 20,000 litres of water to make a litre of biofuel, while *Croton* trees grow wild and yield about 0.35 litres of oil per kilo of nuts.

Taxonomical Position:

Kingdom: Plantae
Subkingdom: Tracheobionta
Infrakingdom: Streptophyta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Malpighiales
Family: Euphorbiaceae
Subfamily: Crotonoideae
Tribe: Crotoneae
Genus: *Croton L*.
Species: *Croton* bonplandianus

Fig. 1. *Croton bonplandianus*

2. GENUS CROTON

Members of the Genus *Croton* are Deciduous shrubs or small trees, variously stellate hairy or with lepidote scales. Stipules minute. Leaves alternate; blades with 2 glands at base. Flowers monoecious, dioecious or a combination of both. Inflorescence racemose, usually terminal. Flowers 5-merous, males with small disk glands, stamens 5-30; females often with vestigial petals; ovary 3-locular. Fruit a capsule. Seeds ecarunculate or caruncle small.

2.1 Phytochemical Aspect of *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 benzyls are oquinoline 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 an 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 this *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 (Table 1). 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). Plant and leaves contain alkaloids, sparsiflorine, crotoflorine, crotsparine, crotosparinine, proporphine, isoquinoline dionone, N-methylcrotsparine and N-methylcrotsparinine Leaves and stem contains β-sitosterol and taraxerol, vomifoliol, uric acid and tetrahydroglazievine. Leaves also contain Rutin. (C18 H 36O 19). 16-Hexadecanoyl hydrazide (88.69%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (5.56%), 2-Piperidinone, N-[4-bromo-n-butyl] (2.56%), Phthalic acid, bis (7-methyloctyl) ester (1.80%) and Phytol (1.39%) were found in leaf. The seed of *Croton bonplandianum* contains diterpines, phorbol ester, including 12-orthotrideconeoly-phorbol-13-acetat (TPA) and myristoyl phorbol acetate (MPA). The roots in addition to ß-sitosterol contain phenolic quinonoid alkaloid norsinoacutine and 3-methoxy-4, 6-dihydroxy morphinan-dien-7-one. An unusual finding of this species is the hyper accumulation of copper in it. The phytocompounds mequinol (0.74%), 4-methylphenol (6.86%) and 3-methylquinoline (0.44%) are present in the latex of *C. bonplandianum*. The fruits of *C. bonplandianum* showed the presence of fifteen major phytocompounds including 9,12,15-Octadecatrienoic acid, methyl ester (z,z,z)- (41.81%), Diazoprogesterone (19.03%), Decanoic acid, ethyl ester (4.86%), 1- Propene, 2- nitro-3-(1-cyclooctenyl) (4.58%) and 6,9,12-Octadecatrienoic acid, 13-Tetradece-11- yn-1-ol (3.47%).

Table 1. Phytochemical analysis of the ethyl acetate leaf extract of *Croton megalocarpus*

Phytochemical	Intensity
Tannins	++
Alkaloids	+++
Flavonoids	+
Saponins	+
Steroid glycosides	+
Triterpenes	-

+Weakly present, ++strongly present, +++Very strongly present, - absent

2.2 Traditional Uses

Croton bonplandianum Baill used traditionally for curing different types of health related problems: gastro intestinal disorders (cholera, boils, bowel complaints, diarrhoea, dysentery, insanity, acute constipation, abdominal dropsy, internal abscesses), respiratory diseases (cold and cough , lungs infection, bronchitis, asthma), hepatic problem (jaundice, liver complaints), analgesic (reduce pain, sprains, headache), it is also used for the treatment of scurvy, malaria, chicken pox, eye diseases**,** skin diseases**,** rheumatism, epilepsy and many other diseases. For Chologogue and purgative this is the most important plant. From the literature it has been recognized and reported that the leaves extract was used for the treatment of cancer, venereal diseases, ulcer and so on.

3 RECENT PHARMACOLOGICAL STUDIES

3.1 Antibacterial Activity

The antibacterial screening of leaf, fruit, latex extracts and fresh latex of *C. bonplandianum* was carried out and 10% w/v test solution of leaf, fruit and latex of *C. bonplandianum* were prepared by dissolving 500 mg of each extract separately in 5 ml of sterile 10% Dimethyl Sulphoxide (DMSO). From this 25, 50, 75 and 100µl extracts contains 2.5, 5, 7.5 and 10 mg, respectively and fresh latex was taken for the analysis of antibacterial activity. The extracts of leaf, fruit and latex of *C. bonplandianum* were loaded at different concentrations (2.5, 5, 7.5 and 10 mg) in the well on pre inoculated Mueller Hinton Agar (MHA) plates with respective bacterial cultures and incubated at 37ºC for 24 hours. Streptomycin (10 µg) was used as a positive control and the solvent 10% DMSO was used as negative control for this study. After incubation, the diameter of the zone of inhibition (mm) around the well was measured using zone reader [12]. The antibacterial activity of the various solvent extracts of the leaf of *C. bonplandianum* against bacterial isolates showed best results at the concentration of 7.5 mg/75 µl and The aqueous leaf extract showed a maximum zone of inhibition 15±2 mm against *S. aureus* while the minimum zone of inhibition 10±1 mm against *P. aeruginosa* when compared to other bacterial isolates. The ethonolic leaf extract showed the highest zone of inhibition 22±2 mm against *E. aerogenes* and *E. coli* while the lowest zone of inhibition 16±2 mm was observed against *E. faecalis* when compared to other bacterial isolates. The acetone extract of leaf showed a maximum zone of inhibition 19±2 mm against *E. aerogenes* and *E. coli* while the minimum zone of inhibition 10±1 mm against *P. aeruginosa*. The chloroform extract of leaf showed 19±2 mm inhibition against *S. aureus* and *E. aerogenes* and the benzene extract of leaf showed 20±2 mm inhibition against *S. aureus* [13].

3.2 Anthelmintic Activity

The anthelmintic activity was performed according to the method. On adult Indian earthworm *Pheretima pothuma* as it has an anatomical and physiological resemblance to the intestinal roundworm parasites of human beings [14]. *Pheretima pothuma* was placed in Petridish containing three different concentrations (20,40,60 mg/ml) each of *Croton bonplandianum* (pet. ether, ethanol, and water extract)solutions. Each petri dish was placed with 6 worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when

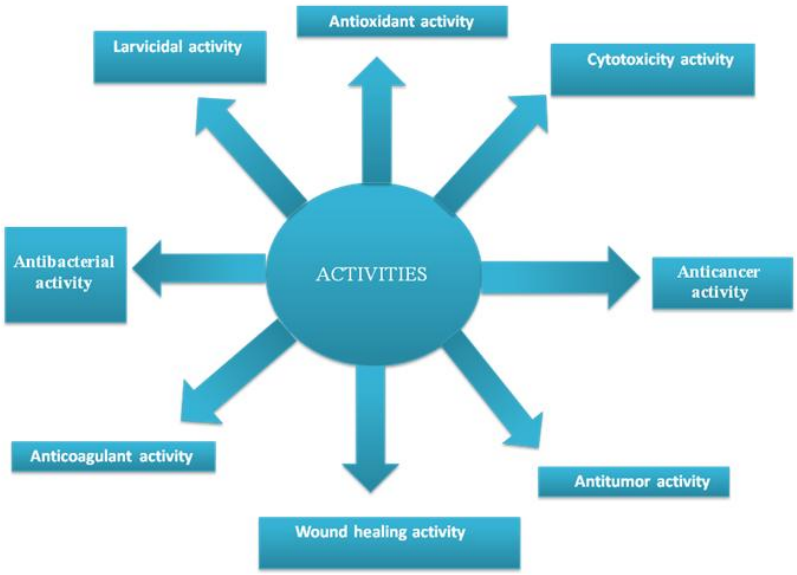

Fig. 2. Role of *Croton bonplandianum* Baill in different activities

given external stimuli. In the same manner, albendazole was included as a reference compound. The Test results were compared with Reference compound Albendazole (20, 40, 60 mg/ml) treated samples [15].

3.3 Antioxidant Activity

3.3.1 Assay for nitric oxide scavenging activity

NO was generated as a result of decomposition of sodium nitroprusside in an aqueous medium, interacts with oxygen at physiological pH to produce nitrite ions, which are measured by using Griess reaction [16,17]. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with Naphthyl ethylenediamine was read at 546 nm and referred to the absorbance of the standard solution of potassium nitrite, treated same way with Griess reagent. The reaction mixture (3ml) containing sodium nitroprusside (10μM) in phosphate buffer and various concentrations of drug i.e., 10, 20, 40, 80, 100 μg/ml of ethanolic extract of *Croton bonplandianum* or ascorbic acid were incubated at 25 degrees C for 120 minutes. Control without test compound kept in an identical manner. After incubation 0.5 ml of the incubation solution was removed and diluted with 0.5 ml of the incubation solution was removed and diluted with 0.5 ml of Griess reagent (1% w/v sulphanilamide, 2% w/v H Po and 0.1% w/v Naphthyl 3 4 ethylene diamine dihydrochloride). The absorbance of the chromophore was read at 546 nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds.

3.4 Anticancer Activity of *Croton bonplandianum*

Croton bonplandianum is a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. The seed oil (croton oil) obtained from this plant or its major active constituent, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), is an irritant and inflammatory agent that has been used widely as a tumor promoter (usual dose = 5-16 nmol, twice a week) on the skin of mice previously initiated with 7,12-dimethylbenz(*a*)anthraceneor other polycyclic aromatic hydrocarbons .TPA at a 10,000-fold lower concentration is an extraordinarily potent stimulator of differentiation in myeloid leukemia cells *in vitro*. In studies with solid tumors, TPA was shown to inhibit the growth, stimulate apoptosis, or enhance differentiation in human tumor cell lines derived from patients with melanoma or prostate, breast, colon, or lung cancer. Treatment of prostate cancer LNCaP cells with clinically achievable concentrations of TPA (1–1.6 nM) resulted in growth inhibition, and treatment of these cells with a several fold higher concentration of TPA caused apoptosis .A synergistic inhibitory effect of TPA and ATRA on the growth of cultured prostate cancer LNCaP cells, and an inhibitory effect of TPAor ATRA administration on the growth of well-established LNCaP tumors in immunodeficient mice were observed. Tumor regressions were observed in several of the treated mice, and administration of a combination of TPA and ATRA to these tumor-bearing mice resulted in a some tumor regression in all of the treated animals .The molecular mechanisms by which TPA and ATRA synergistically inhibit growth and induce apoptosis in LNCaP cells are not known.

3.5 Diphenly-2-picrylhydrazyl (DPPH) Scavenging Effect

Diphenyl-2-picrylhydrazyl (DPPH) forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products [18]. Antioxidant reacts with DPPH and converts it into 1, 1-diphenyl-2-picrylhydrazine free radical. The degree of decolourisation indicates the scavenging potential of the antioxidant drug [19]. 0.1 mm solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of extract solution in ethanol at different concentrations i.e. 10, 20, 40, 80, 100μg/ml. After 30 min absorbance was measured at 517 nm. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using following formula. The antioxidant activity of the extract was expressed as IC. The IC value was 50/ 50 defined as the concentration (μg/ml) of extracts that inhibits the formation of free radicals by 50% [20].

3.6 Antidiabetic and Free Radical Scavenging Activity

Goldie Uppal et al. 2012 discussed the anti-diabetic activity. The ethanol extract of *Euphorbia hirta Linn* was tested using animal screening models. Alloxan administered for 21 days, to induce diabetics. The ethanol extract showed a significantly decreased blood glucose level

(hypoglycemic effect) on alloxan-induced diabetic rats. In vivo and in-vitro study of antidiabetic activity were done by Widharna et al., 2010. From the in-vitro experiment, ethanol extract and ethylacetate fractions had α-glucosidase inhibition activity, while n-hexane, chloroform, butanol and water fractions had no α-glucosidase inhibitory effect. In vivo test also had the same result. Based on in vitro and in vivo test, *Euphorbia hirta* L. ethanolic extract and ethylacetate extract exerted anti-diabetic mechanism and α-glucosidase inhibitory property [21].

3.7 Wound Healing Activity

Wounds are common clinical entities in day to day life, which may be major or minor. The process of wound healing can be classified into five phases, cellular phase (collagenation), narrowing of wound area (wound contraction), collagen deposition (collogenation), epithelial covering (epithelialisation), scar remodelling (cicatrixisation). Wound healing is a process by which damages tissue is restored as closely as possible to its normal state and wound contraction is a process of shrinkage of the area of the wound. The Alcoholic leaf extract of *C. Bonaplandianum* significantly increase the rate of wound concentration, according to Ramachandran et al., it was concluded that herbal extract ointments using *Croton bonaplandianum* ‘s leaf extracts increase the significant rate of wound concentration [22].

3.8 Larvicidal Activity

The results of the larval susceptibility of *A. aegypti* using methanolic leaf extracts of *C. bonplandianum* are presented in the results of the study revealed that the methanolic leaf extract was effective against larvae of mosquito. Furthermore, the effect of larval mortality was observed to be dose-dependent The LC50 and LC90 of the fourth instar of *A. aegypti* were determined to be 123.8 ppm and 364.0 ppm respectively. So leaf of this plant at 124 ppm is suggested for better vector control. The larvicidal property of the leaf extract of, *C. bonplandianum* may be due to the presence of phorbol derivatives, the secondary metabolites of diterpenoids category (Chandel et al., 2005). Maria et al. (2006) reported that the essential oils present in four species of a genus, *Croton* are responsible for their larvicidal activity against the mosquito, *A. aegypti*. Nazer et al. [23] reported that the stem extracts of *C. bonplandianum* was active and significantly lethal against the mosquito, *Culex quinquefasciatus* and he explained that the alkaloids present in the extract had a toxic effect on mosquito larvae.

3.9 Cytotoxicity Activity

Cytotoxicity analyses of silver nanoparticles were performed in A549 and PA1 cell line as per [24]. A549 cells are adenocarcinoma human alveolar basal epithelial cells and PA1 was established from cells taken from ascitic fluid. These two cell lines were maintained in DMEM, supplemented with 2 mM L-glutamine, 1% penicillin, streptomycin, and 10% FCS. Cells were grown at 37ºC in a humidified chamber containing 5% CO2.Exponentially growing cells were harvested from the culture flasks by trypsinization and then resuspended in fresh medium. The suspended cells of 5000 cells/well were dispensed into a 96-well micro-plate and be incubated for 24hrs. Then various concentrations of AgNPs (1, 2.5, 5, 7.5, 10 microgram/ml) were used. Each experiment was conducted in triplicate. The cell viability in the microplate was determined using the MTT (3- (4, 5- dimethylthiazol -2- yle) 2,5-diphenyl-tetraloziumbromide) after incubation [25]. MTT was added to each well 5 mg/ml concentration. After incubation for 4 hrs, the cells from each well were solubilized with 100 µl DMSO for determination of optical density at 570 nm.

3.10 *In vitro* Anti-inflammatory Activity

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the *in vitro* anti-inflammatory activity. Blood was collected from a healthy human volunteer who was not taken any anti-inflammatory drugs for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% NaCl in water) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline. Various concentrations of extracts were prepared (100, 200 and 400 mg/ml) using distilled water and to each concentration 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension were added. It is incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac sodium (5 mg/ml) was used

as the reference standard and a control (distilled water) was prepared to omit the extracts. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100%. The percentage of HRBC membrane stabilization or hemolysis was calculated using the formula: % Inhibition of haemolysis = 100 x [Absorbance of control – Absorbance of test/ Absorbance of Control [26].

3.11 *In-vivo* Anti-inflammatory Activity

The albino rats of either sex were divided into six groups of six animals each. Group- I received 5 ml/kg normal saline p.o. serves as a control group, Group- II, III received 100, 200 mg/kg body weight of extracts of *Jatropha gossypifolia* p.o., and Group- IV, V received 100, 200 mg/kg body weight of extracts of *Croton bonplandianum* p.o., Group- VI received 5 mg/kg of body weight of Diclofenac sodium intraperitoneally taken as a standard. After one hour of the administration of the drugs, acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% (w/v) of freshly prepared suspension of λ-carrageenan in the right hind paw of each rat. The paw volume of the rats was measured in the digital plethysmograph (Ugo basile, Italy), at the end of 60 min., 120 min., 180 min. and 240 min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula, Percentage of inhibition = 100 (1-Vt / Vc) Where Vc is Edema volume in control and Vt is Edema volume in test / standard compound.

3.12 Anticoagulant Activity

K. Raja et al. Blood samples were collected from healthy volunteers, using a disposable polypropylene syringe, and then anti-coagulated using 3.8% trisodium citrate in a polypropylene container (9 parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at 4000 × g for 15 min, and plasma was separated and pooled. The freshly prepared plasma was stored at 4ºC until its use. In a test tube, 0.1 ml test plasma and EDTA were added and shaken briefly to mix the reagent and plasma. The tube was placed at 37ºC for 20 min for incubation. After the incubation, 0.1ml pre-warmed calcium chloride solution was forcibly added into the mixture of plasma and reagent. To this, one ml of aqueous extracts, ethanol extracts and was added separately in different concentrations and kept at 37ºC. A stopwatch was started to record the coagulation time in seconds. The tube was shaken to mix the contents and it was stopped as soon as the clot formation began. The activity is expressed in term of clotting time ratio in relation to control. The steps were repeated three times for each sample, and average of the test value was noted [27]. Normal saline was used in place of the extracts for the negative control, and 50 mg/ml of commercial heparin for the positive control [28]. Effect of aqueous and ethanol extracts of whole plant and leaves on Prothrombin time (PT).

3.13 Antitumor Activity

Methanol twigs extract of *C. bonplandianum* Baill plays an important role in the antitumor activity. It was shown that tumor formation was observed when *Agrobacterium* strains alive on living potato disc. The potato discs were often damaged due to the contamination and other physiological factors when there was no tumor formation. Thus successful attachment of *Agrobacteirum* on living potato disc is needed for the antitumor test of plant extracts. Tumor formation ability of *Agrobacterium* was distinctly inhibited on potato disc in presence of methanol extract. Tumor inhibition was increased with the increasing of concentrations of plant extract. The bioactive compound of this plant may play an important role in developing antitumor drugs for human beings, as there is a similarity between human and plant tumor formation mechanism. This is a rapid, inexpensive, in-house, general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products [29-33].

4. DISCUSSION AND 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. This will yield rich dividends in the coming years. In this present study, an attempt has been made to study the various ayurvedic plants and medicines available in the market for the ailment of the common illnesses affecting the various

systems of our body. About 1800 species are used in classical Indian systems of medicines. The emerging field of herbal products industry holds a great potential for the economic development of the Indian region. There is an increasing trend of using plants as a source of food, medicine and perfumes. It is important to understand that nutraceuticals are nutritionally and medicinally enhanced food with health benefits of recent origin.

The results obtained in this study indicated that the traditional plant, *C. bonplandianum* generally used as a potential source for using drugs like antiinflammatory, anticancer, antimicrobial, insectifuge, nematicide, anti-coronary, 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, twenty-one major phytocompounds were identified in different parts like leaf, fruit and latex of *C. bonplandianum*. Here more research work is required to get rid of this type of infection.

5. CONCLUSION

In conclusion, the results obtained in this study indicated that the traditional plant, *C. bonplandianum* generally used as a potential source for using 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, twenty-one major phyto-compounds were identified in different parts like leaf, fruit and latex of *C. bonplandianum*. Here more research work is required to get rid of this type of infection. Institutions have to cooperate with researchers and scientists to carry on these type of research work. At last but not least people should remain conscious or alert about this and they should have enough knowledge about this infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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