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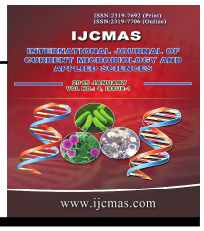

Original Research Article

Antimicrobial activity of *Cassia alata* from Raipur region against clinical and MTCC isolates

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Keywords

Antimicrobial activity, *Cassia alata*, Activity index MTCC, secondary metabolites

A B S T R A C T

The present study reports antimicrobial efficacy of *Cassia alata* against bacterial pathogens. The antimicrobial activity of *Cassia alata* was evaluated against seven clinical and eight MTCC bacterial strains viz., *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*, using agar well diffusion assay. A significant antibacterial activity was observed in acetone extract of root against clinically isolated *P. vulgaris* and *B. subtilis* (MTCC 441) while least activity was recorded in acetone extracts of stem and root against clinically isolated *S. aureus* and *K. pneumoniae* (MTCC 3384) respectively. Activity index of clinical isolates revealed that the acetone extracts of root and leaf were effective against clinically isolated *B. subtilis* and *B. subtilis* (MTCC 441) with reference to streptomycin whereas, the chloroform extract of root was effective for *S. aureus* and acetone extract of root against *B. subtilis* (MTCC 441) with reference to tetracycline. The phytochemical analysis of *Cassia alata* revealed presence of alkaloid, cardioglycoside, fat and oil, flavonoid, gum and mucilage, glycoside, phytosterol, quinone, resin, saponin, tannin and terpanoid. The yield of phytochemicals was more for leaves followed by stem and root.

Introduction

Nature has been a source of wide diversity of medicinal plants and mankind has used many species for centuries since time immemorial for their benefit. The medicinal property of the plants has been exploited for the cure of several ailments (Cragg *et al.*, 1999). Herbal medicines are used by nearly 75-80% of the world population, mainly in developing countries for primary health care because of their compatibility with human body and lesser side effects (Parekh *et al.*, 2005). Chhattisgarh is rich in forest resources containing huge array of medicinal plants and the local tribes are traditionally dependent on such resources (Ekka, 2011). Compounds produced by plants are of interest as sources of safe or more effective substitutes for synthetically

produced antimicrobial agents (Sarkar *et al.*, 2013).

Antibiotics were discovered to provide the source for the therapy of microbial infections. Excessive use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains (Singh *et al.*, 2010). The emergence and spread of antimicrobial resistance is a growing problem of the globe and threat to become a universal crisis. Infectious diseases caused by these multidrug resistant strains remain the leading cause of death. Thus people are turning their attention to alternative novel antimicrobial agents to combat such pathogens (Dehpour *et al.*, 2011; Jain *et al.*, 2011).

Phytochemicals possess disease curative potentiality by inhibiting the growth of pathogenic microorganisms (Olalde, 2005; Renu, 2005). Phytochemicals are biologically active compounds derived from plant physiological processes and are, responsible for conferring colour, flavor, smell, texture and several biological properties including antimicrobial property to the plants (Kumar *et al.*, 2007). The medicinal properties in plants, due to the presence of secondary metabolites, serve for plant defense against predation by microorganisms, insects and herbivores (Hagg *et al.*, 2013).

India is rich in all the three levels of biodiversities including species diversity, genetic diversity and habitat diversity (Samant *et al.*, 2007). Considering the vast potentiality of medicinal plants as antimicrobial agents the present investigation was undertaken to assess the antimicrobial activity of *Cassia alata* Linn. (family Caesalpiniaceae). It is an ornamental shrub, commonly known as "candle tree". The plant has been reported for curing dysentery, scabies, ulcers, helminthic infection and stomach disorder (Abubacker and Kumar, 2007; Doughari and Okafor, 2007). This study is first to explore about the antimicrobial activity and phytochemical analysis of *Cassia alata* found in this region.

Materials and Methods

Plant sample collection

The healthy leaf, stem and root of *Cassia alata* were collected from the village Raipura (Latitude 21.21° N and Longitude 81.59° E) and brought to the laboratory. Different parts of the plants were washed separately with tap water and shade dried at room temperature to attain constant weight. The air dried samples were powdered in an electric blender and stored in plastic bags for further use.

Extraction

The dried powder material was extracted sequentially in four different solvents based on their polarity index viz., chloroform, acetone, methanol and aqueous. 15 g powdered material was extracted first with 150 ml of chloroform (non-polar), followed by acetone (dipolar), methanol (polar) and aqueous (polar) using soxhlet apparatus for 6-8 hours. The crude extract obtained was concentrated in an incubator at 40°C until the solvent evaporated completely and later stored at 4°C till use.

Preparation of stock solution

The crude extract of the plant material was dissolved in 50% DMSO to prepare a stock solution.

Test microorganism

The clinical isolates *viz., Bacillus subtilis,*

Bacillus cereus, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus* and *Staphylococcus epidermis* were procured from Pt. JLN Medical College, Raipur for antibacterial testing. Likewise MTCC isolates *viz*., *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96), *Staphylococcus epidermidis* (MTCC-435), *Proteus vulgaris* (MTCC-744), *Escherichia coli* (MTCC-1687), *Klebsiella pneumoniae* (MTCC-3384), *Pseudomonas aeruginosa* (MTCC-741) were procured from IMTECH, Chandigarh.

Inoculum preparation

24 hr cultures were optimized by adjusting the optical density of the culture broth with normal saline to required OD of 0.08 at 620 nm using a spectrophotometer which was equivalent to 10^8 cfu/ ml (Basri and Fan, 2005).

Antimicrobial activity

The antibacterial assay of the extracts was performed using agar well diffusion method (Sen and Batra, 2012). The 200 µl of inoculum was spread on Muller Hinton agar (Hi-media) plate using a sterilized swab. 5 mm well was bored in the plate and filled with 20 µl of crude test extract. Cultures with standard antibiotic, streptomycin and tetracycline with a concentration of 10µg/ 20µl were simultaneously maintained. The zone of inhibition was measured in mm and expressed as Mean ± Standard Error (SE).

Activity index

This was calculated by dividing diameter of inhibition zone with extract to diameter of the zone of standard antibiotic (Usman *et al.,* 2007).

Qualitative phytochemical analysis

Phytochemical analysis was carried out on the extracts of leaf, stem and root of *C. alata* in order to determine the presence of varied secondary metabolites *viz*., alkaloids, cardioglycoside, fat and oil, flavonoid, gum and mucilage, glycoside, phytosterol, quinone, resin, saponin, tannin and terpanoid following Harborne (1998).

Results and Discussion

The antimicrobial activity of acetone extract of root was highest on *P. vulgaris* with zone of inhibition of 28.0±0.5 mm and on *B. subtilis* (MTCC 441) with zone of inhibition of 22.3±0.3 mm, while the lowest activity was noticed with acetone extract of stem against *S. aureus* with zone of inhibition of 8.0±0.5 mm. Acetone extract of root gave a zone of inhibition of 8.3±0.6 against *K. pneumonia* (MTCC 3384) (Figure 1, 2, 3a and b). No significant antimicrobial activity was observed in the aqueous extracts.

The activity index for clinical isolates was pragmatic with reference to streptomycin. The highest activity index was recorded with acetone extract of root for *B. subtilis* and the least activity index was observed in acetone extract of stem against *S. aureus*. With tetracycline, highest activity index was recorded in chloroform extract of root against *S. aureus* and least activity index was noted for *P. vulgaris*. Similar pattern was observed with MTCC isolates with reference to streptomycin. Acetone extract of leaf recorded highest activity index against *B. subtilis* while least activity index was noted for methanol extract of leaf against *B. subtilis*. However highest activity index was observed for acetone extract of root for *B. subtilis* and least in chloroform extract of root for *S. aureus* with reference to tetracycline (Figure: 4 and 5).

The leaf extracts of *Cassia alata* gave a total yield of 42.26% followed by stem extract (25.40%) and root extract (14.50%) respectively (Figure 6). The phytochemical analysis revealed presence of several secondary metabolites viz., alkaloids, cardioglycoside, fat and oil, flavonoid, gum and mucilage, glycoside, phytosterol, quinone, resin, saponin, tannin and terpanoid in chloroform, acetone, methanol and aqueous extracts of *C. alata* (Table 1).

The medicinal plants are used in traditional treatment to cure variety of diseases and against various bacterial infections. The antimicrobial activity of *C. alata* against gram positive and gram negative bacteria, such as, *B. subtilis, B. cereus, S. aureus, S. epidermidis, E. coli, K. pneumoniae, P. aeruginosa* and *P. vulgaris* revealed that the gram positive bacteria were more susceptible towards all the extracts as compared to gram negative clinical and MTCC isolates. The clinical isolates were found to be more susceptible towards the extracts as compared to MTCC isolates. The reason for the dissimilar sensitivity between gram positive and gram negative bacteria could be attributed to the morphological differences in the cell wall between these microorganisms. Gram negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes (Gupta *et al.*, 2009). In present investigation the antibacterial activity in acetone extract of root of *C. alata* exhibited highest zone of inhibition against *P. vulgaris* and *B. subtilis* (MTCC 441).

Activity index for clinical isolates in case of acetone extract of root was highest for *B. subtilis* while least in acetone extract of stem for *S. aureus* with reference to streptomycin. The chloroform extracts of root with reference to tetracycline, showed highest activity index for *S. aureus* whereas, least in the methanol extract of leaf for *P. vulgaris*. Similar findings were also reported by Khan (2009). The activity index of acetone extract of leaf of *C. alata* was recorded highest against MTCC *B. subtilis* isolates and the least activity index was observed for methanol extract of leaf against *B. subtilis* with respect to streptomycin, whereas, with reference to tetracycline highest activity index was observed for acetone extract of root for *B. subtilis* and least in chloroform extract of root for *S. aureus*.

The highest yield of the extracts recorded in aqueous followed by methanol, acetone and chloroform was based on the solubility index of phytoconstituents in different solvents (Manivannan *et al.*, 2010; Pandey and Gupta, 2013). Among all the three parts under study leaf exhibited highest percentage yield (42.26%) followed by stem (25.40%) and root (14.50%), respectively. Similar observations were also reported by (Doughari and Okafor, 2007; Tomar *et al.*, 2009).

In *C. alata* extracts the presence of several phytochemicals such as alkaloids, cardioglycosides, fats and oils, flavonoids, glycosides, phytosterols, quinone, resins, tannins and terpanoids was detected, which might be responsible for antimicrobial activity. El-mahmood and Doughari (2008) reported the presence of these phytochemicals in *C. alata* Linn. Extracts of *C. alata* in this study demonstrated a wide spectrum of activity against gram positive bacteria. The broad-spectrum antibacterial activity of the plant extracts could be attributed to the presence of varied phytoconstituents in it (Mahesh and Satish, 2008; Pandey and Gupta, 2014). The bioactive compounds from this plant can therefore be employed in the formulation of

antimicrobial agents for the treatment of various bacterial infections. Since this being a preliminary screening study of crude extracts and its further isolation, identification and purification would open a new avenue for formulating novel chemotherapeutic agents which could be the future direction for subsequent investigation.

Table.1 Phytochemical analysis of leaf, stem and root extracts of *Cassia alata*

S.N.	Phytochemical	Chloroform			Acetone			Methanol			Aqueous		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
1	Alkaloid	-	-	-	-	-	-	+	+	+	+	+	+
2	Cardio glycoside	-	-	-	+	+	+	-	-	-	-	-	-
3	Fat & oil	+	+	+	+	+	+	-	-	-	-	-	-
4	Flavonoid	-	-	-	+	+	+	+	+	+	+	+	+
5	Gums & mucilage	-	-	-	-	-	-	-	-	-	+	+	+
6	Glycoside	+	+	+	+	+	+	+	+	+	+	+	-
7	Phytosterol	+	+	+	+	+	+	+	+	+	-	-	-
8	Quinone	+	+	+	+	+	+	+	+	+	+	+	+
9	Resin	+	+	+	+	+	+	-	-	-	-	-	-
10	Saponin	-	-	-	-	-	-	-	-	-	+	+	+
11	Tannin	-	-	-	+	+	+	+	+	+	+	+	+
12	Terpenoid	-	-	-	+	+	+	+	+	+	-	-	-

Figure.1 Antimicrobial activity of *Cassia alata* against clinical isolates

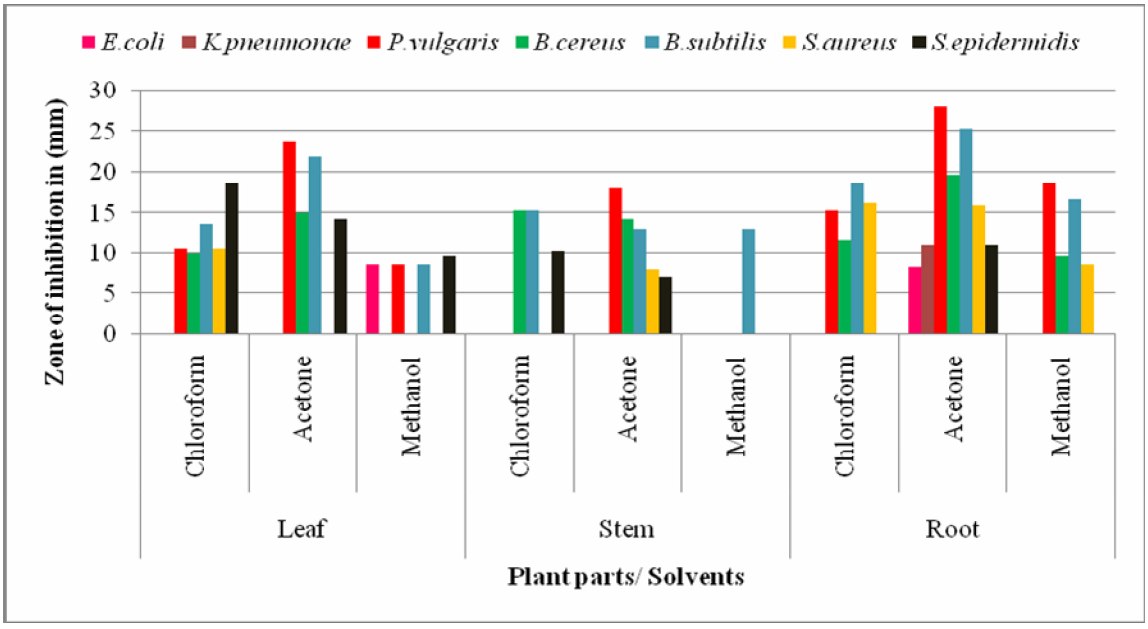


Figure.2 Antimicrobial activity of *Cassia alata* against MTCC isolates

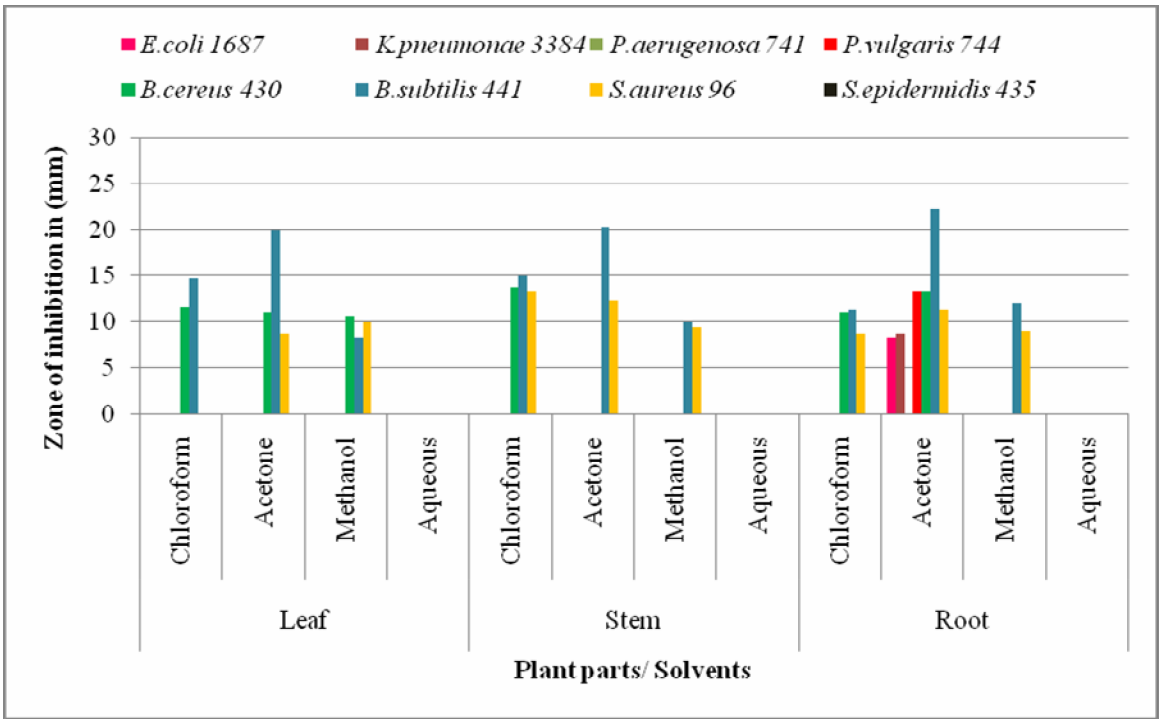


Figure.3(a) Zone of inhibition with acetone extract of stem of *C. alata* against *B. cereus* (MTCC 430)

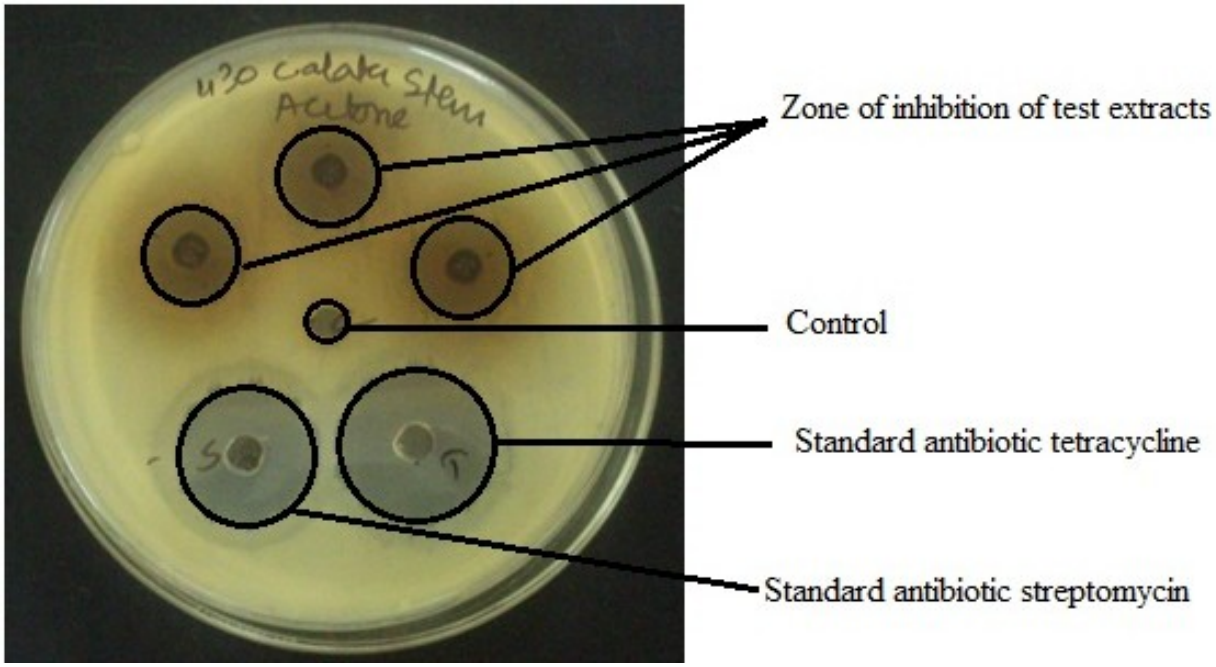


Figure.3(b) Zone of inhibition with acetone extract of root of *C. alata* against *S. aureus*

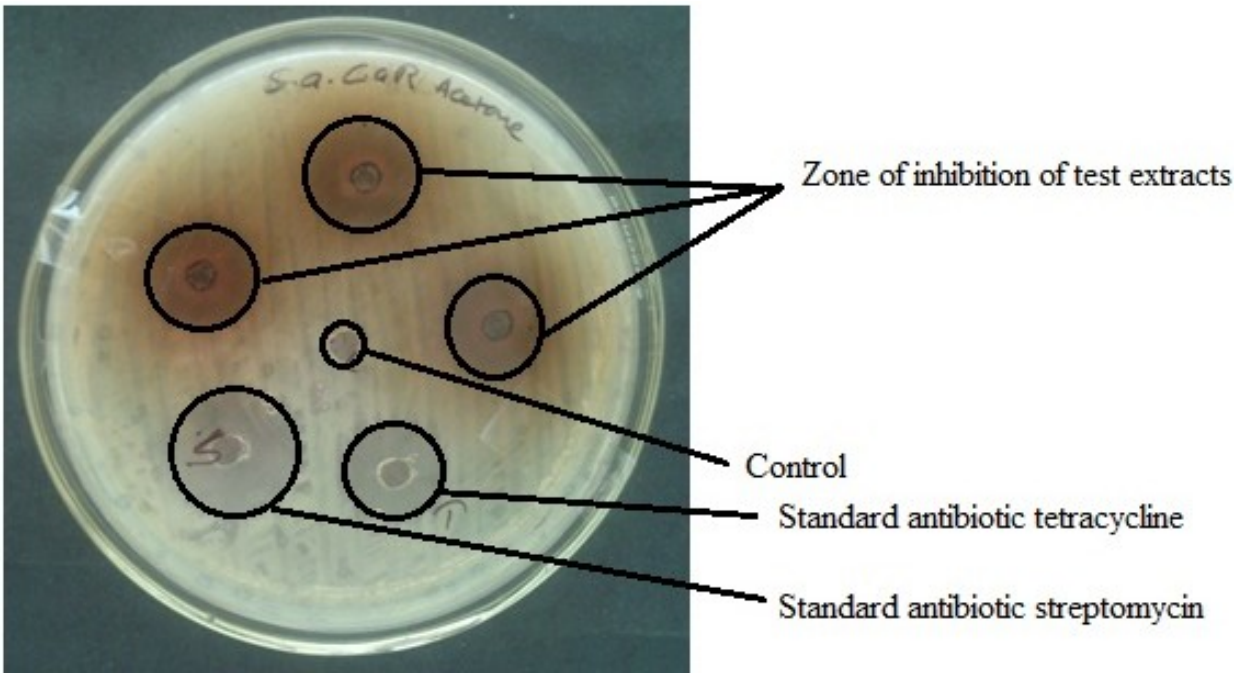
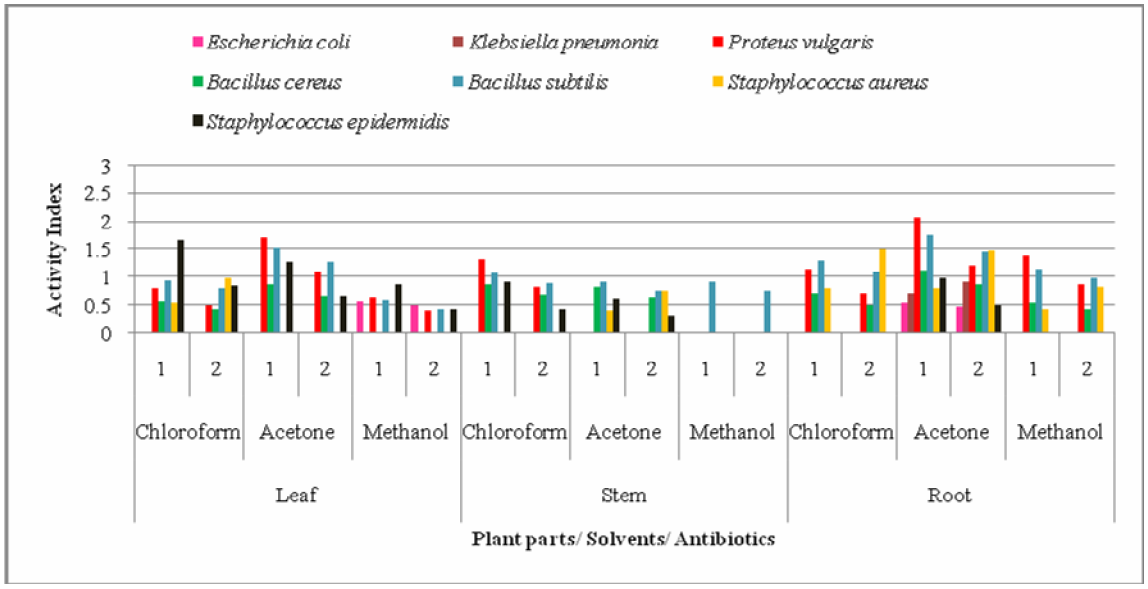
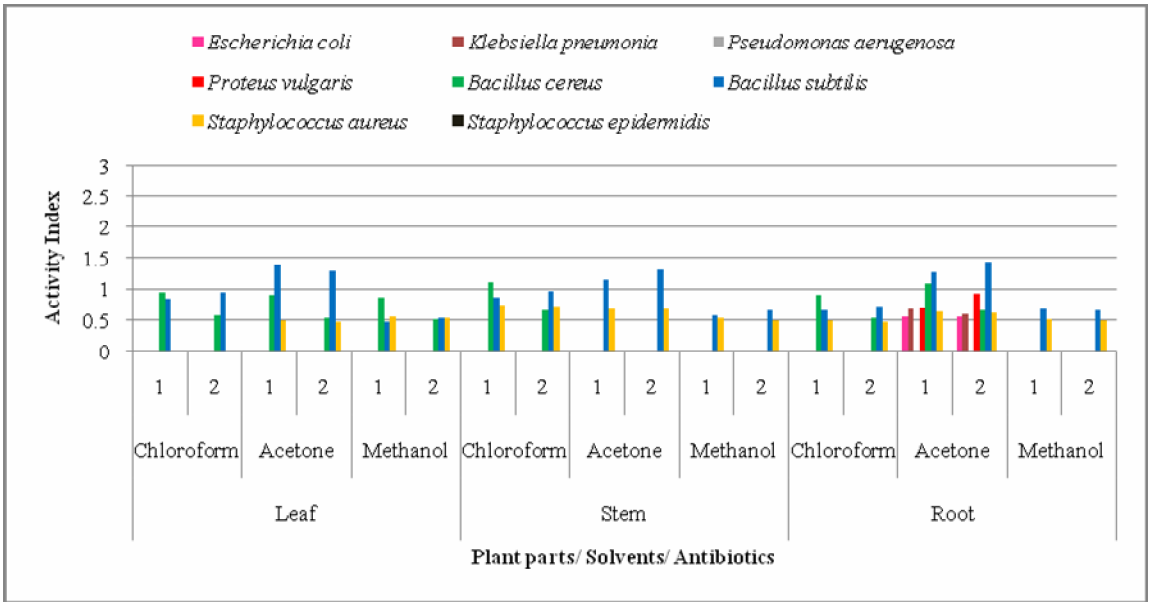


Figure.4 Activity index of *Cassia alata* leaf, stem and root extracts against clinical isolates with reference to streptomycin and tetracycline



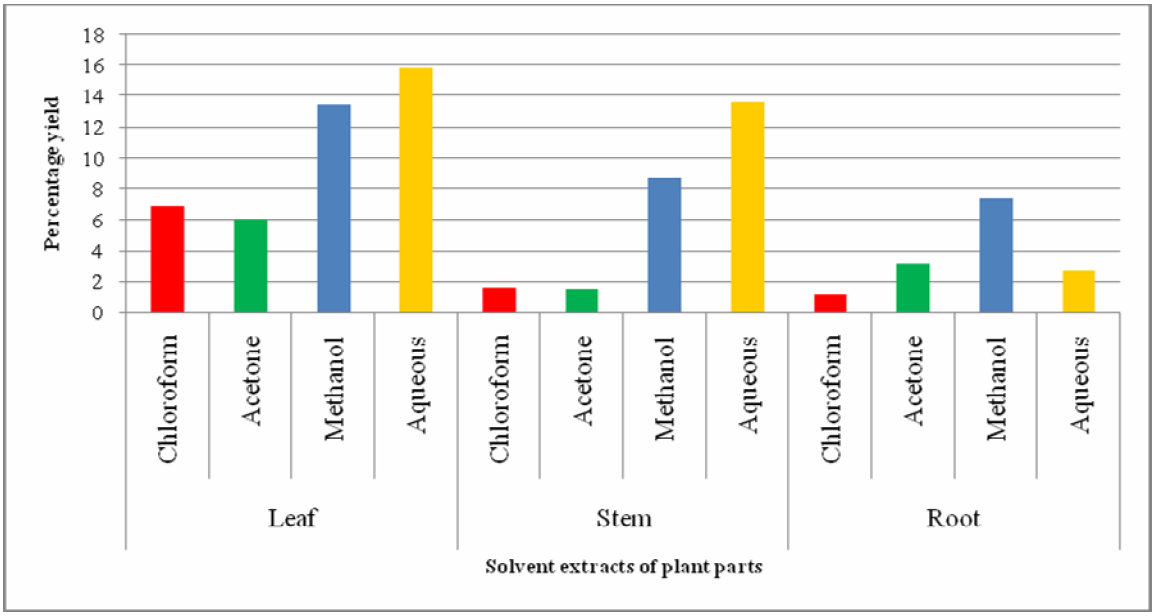
Standard antibiotics: 1. Streptomycin, 2. Tetracycline

Figure.5 Activity index of *Cassia alata* leaf, stem and root extracts against MTCC isolates with reference to streptomycin and tetracycline



Standard antibiotics: 1. Streptomycin, 2. Tetracycline

Figure.6 Yield of *Cassia alata* in various solvents



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