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G Chaitanya
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

Ch Pavani
(1) Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India
(2) Biotechnology Department, Singareni Collieries Women's Degree College, Kothagudem, Telangana State, India

V Suvarchala
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

D Sunitha Bai
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

Spoorthi
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

D Ramakrishna
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

T Shasthree
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

Corresponding Author:
T Shasthree
Plant Tissue and Organ Culture and Environmental Plant Physiology Laboratory, Department of Biotechnology, Kakatiya University, Warangal, Telangana, India

In vitro antimicrobial activity of leaf, stem fruit and root crude extracts of *Momordica cymbalaria* Fenzl: A medicinally important cucurbit

G Chaitanya, Ch Pavani, V Suvarchala, D Sunitha Bai, Spoorthi, D Ramakrishna and T Shasthree

Abstract
Medicinal plants are the best source for the worldwide synthetic drug industries. The compounds extracted from the medicinal plants contain several antimicrobial properties. It is necessary to systematically test plants for the detection of antimicrobial compounds to work on microbial pathogens for new drug development. The plant *Momordica cymbalaria* Fenzl. is a medicinally important cucurbit, which contains secondary metabolites like phenols, steroids, flavonoids, alkaloids, tannins and glycosides. Extracts of *Momordica cymbalaria* leaf, stem, fruit and roots were screened for the antimicrobial activity by using agar disc diffusion method. Streptomycin is used as a standard antibiotic and different types of solvents such as methanol, ethanol, chloroform, hexane and water were used for the antimicrobial activity. Among all the solvents methanol showed high antimicrobial properties against *Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* bacteria.

Keywords: *Momordica cymbalaria,* streptomycin, methanol extract, *Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*

Introduction
Environment and humans are closely interconnected. Researchers point out the evidence, which is indicating the crucial role of microbial ecosystems in maintaining the insubstantial balance between nature and human health (McCann, 2000) [11]. Since many bacteria showing resistance to standard medicines are increasing frequently, medicinal plants constitute an alternative to treatment infection, which comprises the important aspect that have antimicrobial action and are symbiotic with currently available antimicrobial drugs. Scientifically known medicinal plants were subjected to biological tests and a number of new antibiotics were introduced into the market.

In ancient times, initially plant crude form was used in traditional folk healing therapies or for other diseases, now these plant extracts are used in modern drugs which are commercially proven for several diseases. The major benefits of using plant derived medicines are offers cost free treatments and more affordable therapeutic benefits, and also safer than synthetic drugs (Aboaba *et al*., 2011) [1].

Many cultural, and some non-cultural pathogens, produce various substances that inhibit other organisms that grow in their place. If we consider antibiotics as secondary metabolites of microorganisms, it reduces the field to certain microorganisms such as Penicillium, Streptomyces species and Bacillus species (Hardoim *et al*., 2015) [8]. The organism is affected by antibiotics produced by other organisms, but shows resistance to their own antibiotic action, which is effective against closely related species. In many cases, it is not even known how or why bacteria become resistant to won antibiotics, but it may be worthwhile to study and gather such information from the cellular and molecular basis of drug resistance design studies (Nash, 2015) [14].

Antimicrobial activity of a particular compound is known as the intrinsic capacity of substances like drugs or toxins to change one or more chemical or physiological functions of a cell, tissue, organ or organism. Jackson *et al*., 2007 [10] described Biological activity is the capacity of a specific molecular entity to achieve a specific biological effect on a target. Along with this activity, its concentration and duration of cellular exposure to substance is also determined. Biological activity may reflect "domino effect," in which the modification of one function interrupts the normal activity of one or more other functions. Secondary metabolite success depends on the presence of high diversity of chemicals and wide spectrum of biological activities; it possesses a high amount of nutrients, alkaloids, polyphenols and

phytoestrogens. These secondary metabolites have been established in the treatment and prevention of various diseases such as cancer, inflammation of liver, diabetes, and cardiovascular injuries (Shivaraj *et al*., 2011) [25].

Screening of medicinal plants for antibacterial activities and phytochemical is important to find out the potential new compounds for therapeutic use. For example, latex of some species of Ficus is exploited in traditional folk medicine for its parasiticidal property, anthelmintic activity, antischistosomal activity in South and Central America (Pistelli *et al*., 2000; Cowman *et al*., 1999; Ahmed *et al*., 2014) [20, 3, 1]. In nature, biodiversity contains a wide range of diverse chemical structures with potentially active new molecules having favorable biological activities. Such new potential molecules can be used as chemical templates in designing and the synthesis of novel drugs. The historical studies on plants have proven that plants serve as rich sources of molecules with high therapeutic potential and many major current drugs are plant-derived compounds (Newman and Cragg, 2016) [16].

Several species of Cucurbitaceae family have been tested by the worldwide scientists for biological activity studies on the interactions between natural products of plants and antimicrobial drugs. These types of research have multiplied in recent years, indicating the importance and explain those types of interactions, which can be favorable or harmful. Though, such associations, even if beneficial, they are not necessarily used in the treatment of infectious diseases because more studies are needed, especially *in vivo* and *in vitro* studies and research on the toxicity of these products to humans (Silva NCC and Fernandes Júnior A, 2010) [24].

Momordica cymbalaria Fenzl. is a medically important cucurbit. It is recognized in traditional medicine, used for the treating diabetes mellitus, rheumatism, diarrhea, skin diseases and ulcer (Jeyadevi *et al*., 2012) [9]. The fruits and leaves were used as a vegetable (Kirtikar and Basu, 1933). The fruit contains citric acid, maleic acid, vitamin C and high crude fiber content (Parvathi and Kumar, 2002) [18], and also saponins, phenols, steroids, terpenoids and flavonoids like quercetin (Pallavi *et al*., 2017) [17]. The plant part extracts shows anti-diabetic (Firdous *et al*., 2009) [7], hypolipidemic (Ezra *et al*., 2014) [6] , anti-diarrheal and anti-microbial (Vrushabendra swamy *et al*., 2008), anti-ulcer, neuroprotective (Dhasan *et al*., 2010) [5] , cardioprotective, anti-ovulatory and abortifacient (Raju *et al*., 2008), hepatoprotective, nephroprotective, anti-cancer (Nagaratana *et al*., 2016) [14], antioxidant (Prashanth *et al*., 2013) [21] activities. Hence, in our study we are discussing the antimicrobial activity of *Momordica cymbalaria* leaf, stem, fruit and root extracts in different solvents.

Materials and Methods

Preparation of Plant extracts

Leaf, stem, fruit and roots of *Momordica cymbalaria* collected and kept for shade dried for 10- 15 days. After drying the plant parts they were chopped into small pieces and grinded into fine powder. 5 gr of leaf, stem, fruit and root powder each were taken and dissolved in 30ml of different solvents such as methanol, ethanol, chloroform, hexane and water. The resulting extracts were kept in a rotary shaker for 48 hrs at 28 °C. After 48 hrs these extracts were filtered and poured in sterile bowls and kept for 2-3 days of drying in the oven. After drying the extracts were collected in the air tight apendoffs and stored at 4 °C and used for the antimicrobial activity.

The antimicrobial activity of plant extracts with different solvents such as methanol, ethanol, chloroform, hexane and aqueous was determined using agar diffusion method using clinically important pathogens containing Gram-positive and Gram-negative test strains. All the pure bacterial cultures were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The pure cultures of the bacterial strains such as, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* were taken from the 24 hr old bacterial suspension cultures. 200mg of bacterial culture spread uniformly onto the Luria-Bertani agar (LB) medium (20ml) each containing petri dishes using sterile spreaders. After spreading the bacterial cultures, five hollow blocks of medium (6 mm) were cut from the LB media plates using 1 ml sterile pipette tips. Plant extracts such as leaf, stem, fruit and root extracts (25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml) tested against bacteria to analyze the antimicrobial activity. Streptomycin antibiotic (10µg/ml) was used as a positive control for antimicrobial activity. The bacterial culture-inoculated on petri plates treated with plant extracts and streptomycin was incubated for 24 hr at 37 °C for the observation and measuring of the inhibition zone. The experiment was carried out in triplicates.

Results and Discussion

Qualitative screening of antibacterial assay was screened by agar well diffusion method. The results revealed the anti microbial activity in all leaf, stem, fruit and root extracts of *Momordica cymbalaria.* Out of five solvent extracts used, methanol plant extracts of *M. cymbalaria* show highest inhibitory zones against gram positive and gram negative bacteria followed by ethanol, chloroform, hexane and aqueous extracts. Sajjan sangeeta *et al*., (2010) [25] reported that *Momordica cymbalaria* aerial plant parts, ethanol and aqueous extracts show high inhibitory zones against *E.coli*, *S. aureus*, and *P. aeruginosa* strains.

Effect of leaf methanolic extract of *M. cymbalaria* on bacterial activity

In leaf extracts, methanol solvent extracts showed highest results compared with remaining ethanol, chloroform, hexane and aqueous extracts. Methanol leaf extract against *E. coli* showed a high inhibition zone when comparing with remaining *S. pneumonia*, *S. aureus* and *P. aeruginosa* strains (fig.1). When the leaf methanol extracts were treated against *S.aureus*, low concentration (25µg/ml) of leaf methanol extract did not show any inhibition zone. When the leaf methanol extract concentration increases, the zone of inhibition also increases (fig.1). Leaf methanol extract treated against *S. pneumonia*e and *P. aeruginosa* low concentration to high concentration of leaf extracts shows gradually increased inhibitory zone. The high inhibition activity was exhibited against *P. fluorescens*, followed by *B. sphaericus* in *Solanum khasianum* leaf methanol extract (Pavani and Shasthree, 2021) [19].

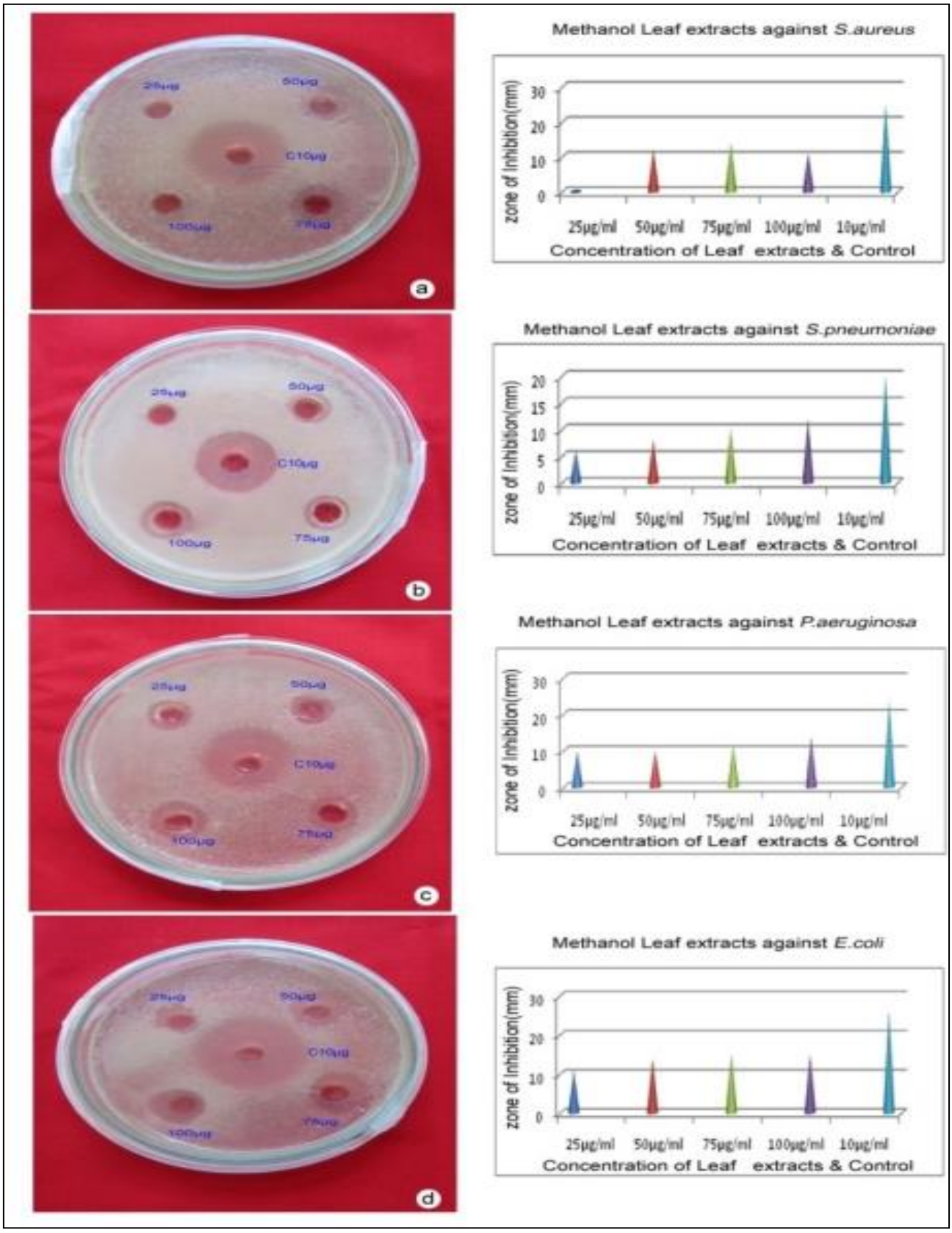

Fig 1: Bacterial activity of methanol leaf extract of *Momordica cymbalaria* Fenzl. (a). Inhibition zone of methanol leaf extract and streptomycin (control) against *Staphylococcus aureus*. (b) Inhibition zone of methanol leaf extract and streptomycin (control) against *Streptococcus pnemoniae*. (c) Inhibition zone of methanol leaf extract and streptomycin (control) against *Pseudomonas aerguniosa*. (d) Inhibition zone of methanol leaf extract and streptomycin (control) against *Escherichia coli*

Effect of stem methanolic extract of *M. cymbalaria* on bacterial activity

In stem extracts, methanol solvent extracts were shown to have the highest results compared with remaining ethanol, chloroform, hexane and aqueous extracts. Methanol stem extracts against *S. pneumoniae* showed a high inhibition zone when comparing with remaining *S. aureus*, *P. aeruginosa* and *E.coli* strains. Similar results were reported by Sajjan (2010) [25]. At high concentration (100µg/ml) of stem extracts against all four bacteria strains shows high inhibitory zones when comparing with low concentration of stem extracts (fig.2).

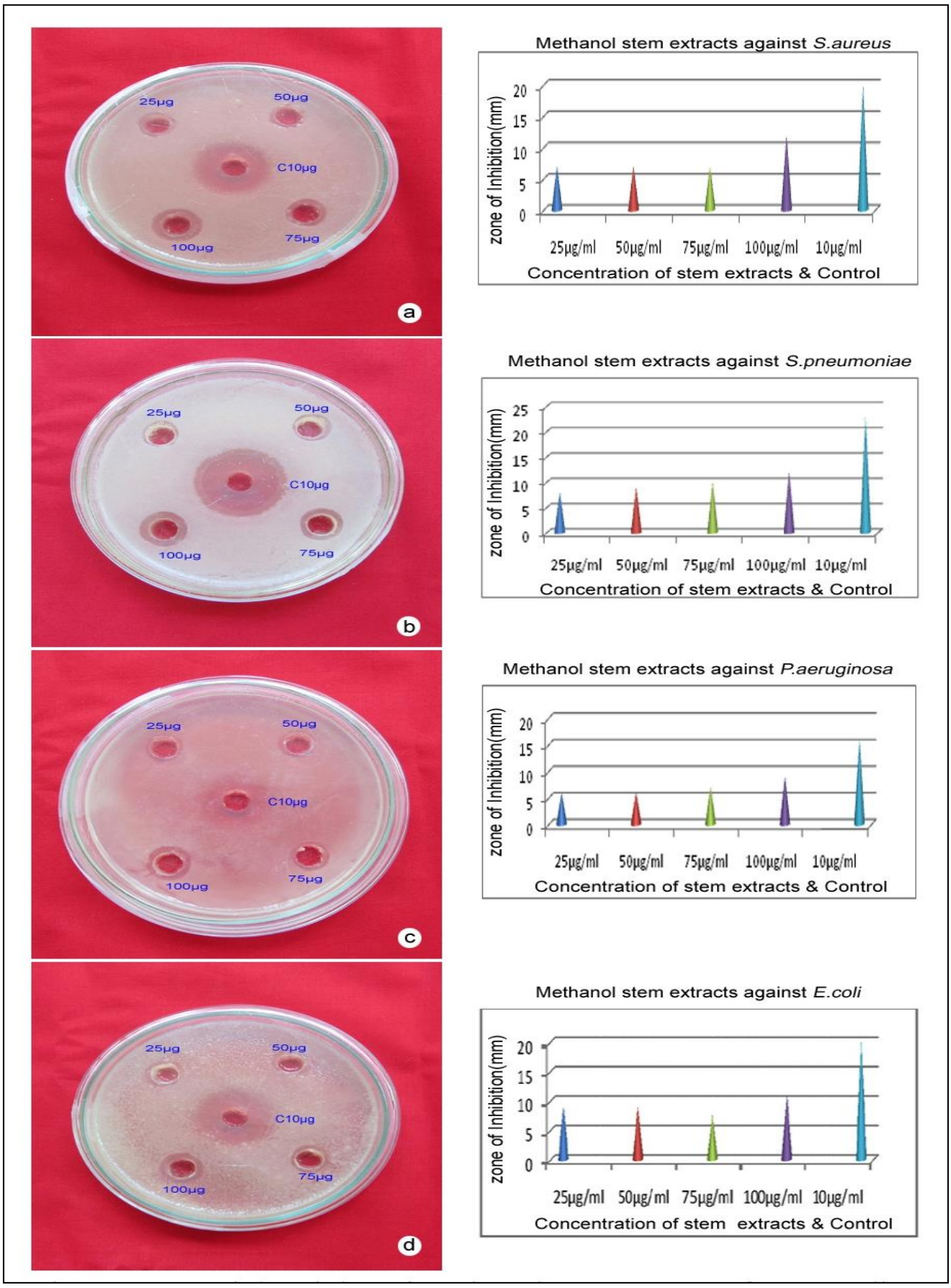

Fig 2: Bacterial activity of methanol stem extract of *Momordica cymbalaria* Fenzl. (a) Inhibition zone of methanol stem extract and streptomycin (control) against *Staphylococcus aureus*. (b) Inhibition zone of methanol stem extract and streptomycin (control) against *Streptococcus pnemoniae*. (c) Inhibition zone of methanol stem extract and streptomycin (control) against *Pseudomonas aerguniosa*. (d) Inhibition zone of methanol stem extract and streptomycin (control) against *Escherichia coli*.

Effect of fruit methanolic extract of *M. cymbalaria* on bacterial activity

In fruit extracts, methanol solvent extracts were shown to have the highest results compared with remaining ethanol, chloroform, hexane and aqueous extracts. Similar results were reported by Vrushabendra swamy (2007) [26] in *Momordica cymbalaria*. Methanol fruit extracts against *S. pneumoniae*, low concentration (25µg/ml) of fruit methanol extracts did not show any sensitivity against *S. pneumoniae* (fig.3) but at high concentrations resulted with inhibitory zones. Methanol fruit extracts against *P. aeruginosa* showed highest inhibitory zones at low concentrations to high concentration of fruit extracts when comparing with other treated bacteria.

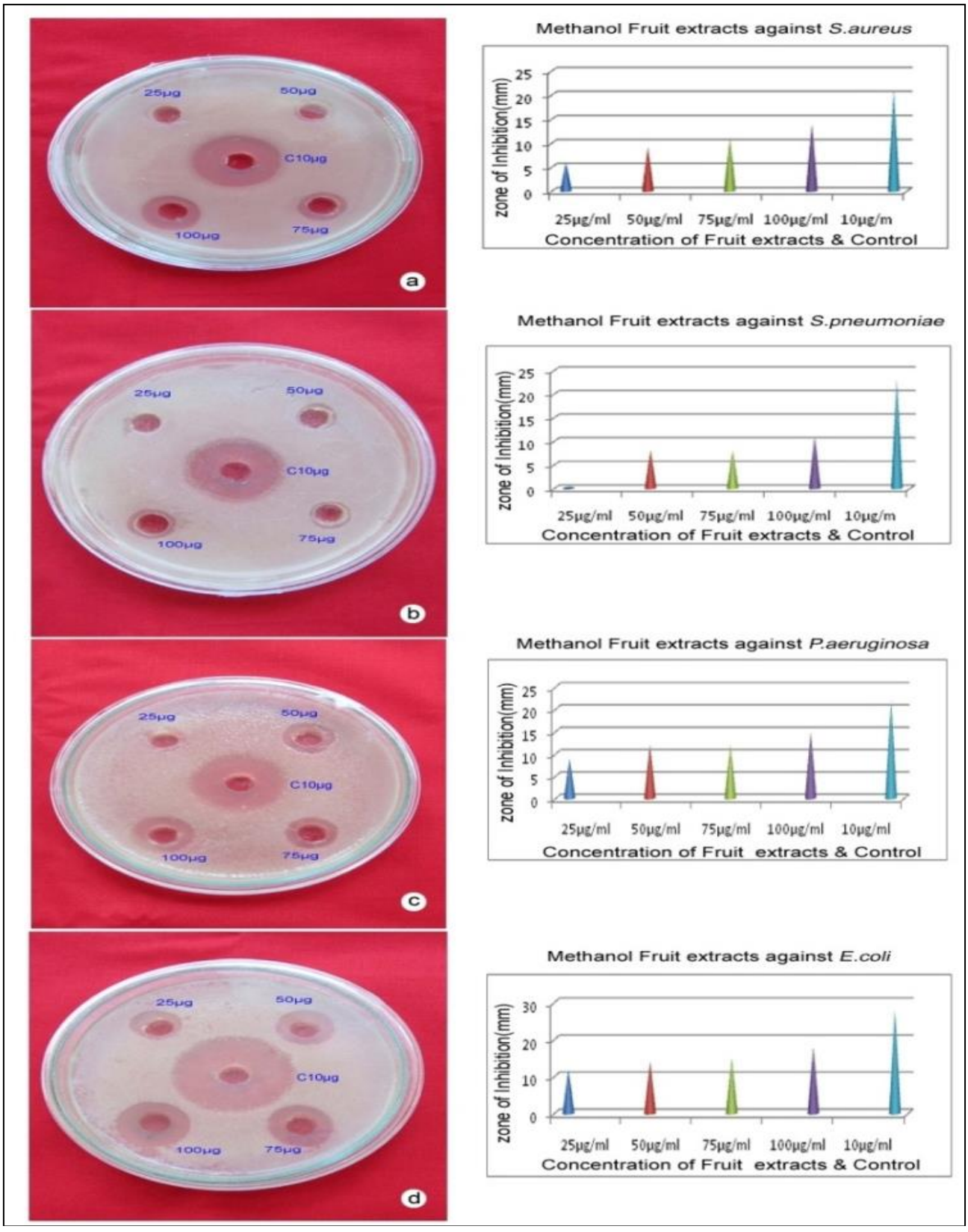

Fig 3: Bacterial activity of methanol fruit extract of *Momordica cymbalaria* Fenzl. (a) Inhibition zone of methanol fruit extract and streptomycin (control) against *Staphylococcus aureus*. (b) Inhibition zone of methanol fruit extract and streptomycin (control) against *Streptococcus pnemoniae*. (c) Inhibition zone of methanol fruit extract and streptomycin (control) against *Pseudomonas aerguniosa*. (d) Inhibition zone of methanol fruit extract and streptomycin (control) against *Escherichia coli*.

Effect of root methanolic extract of *M. cymbalaria* on bacterial activity

In root extracts, methanol solvent extracts were shown to have the highest results compared with remaining ethanol, chloroform, hexane and aqueous extracts. Leaf methanol solvent extracts showed sensitivity against all treated gram positive and gram negative bacteria. Among four bacteria, *E.coli* showed high inhibitory zones when treated with low to high concentration of methanol root extracts (fig.4). In *Momordica charantia* methanol plant extracts showed high sensitivity against *S. aureus* (Mwambete, 2009) [12].

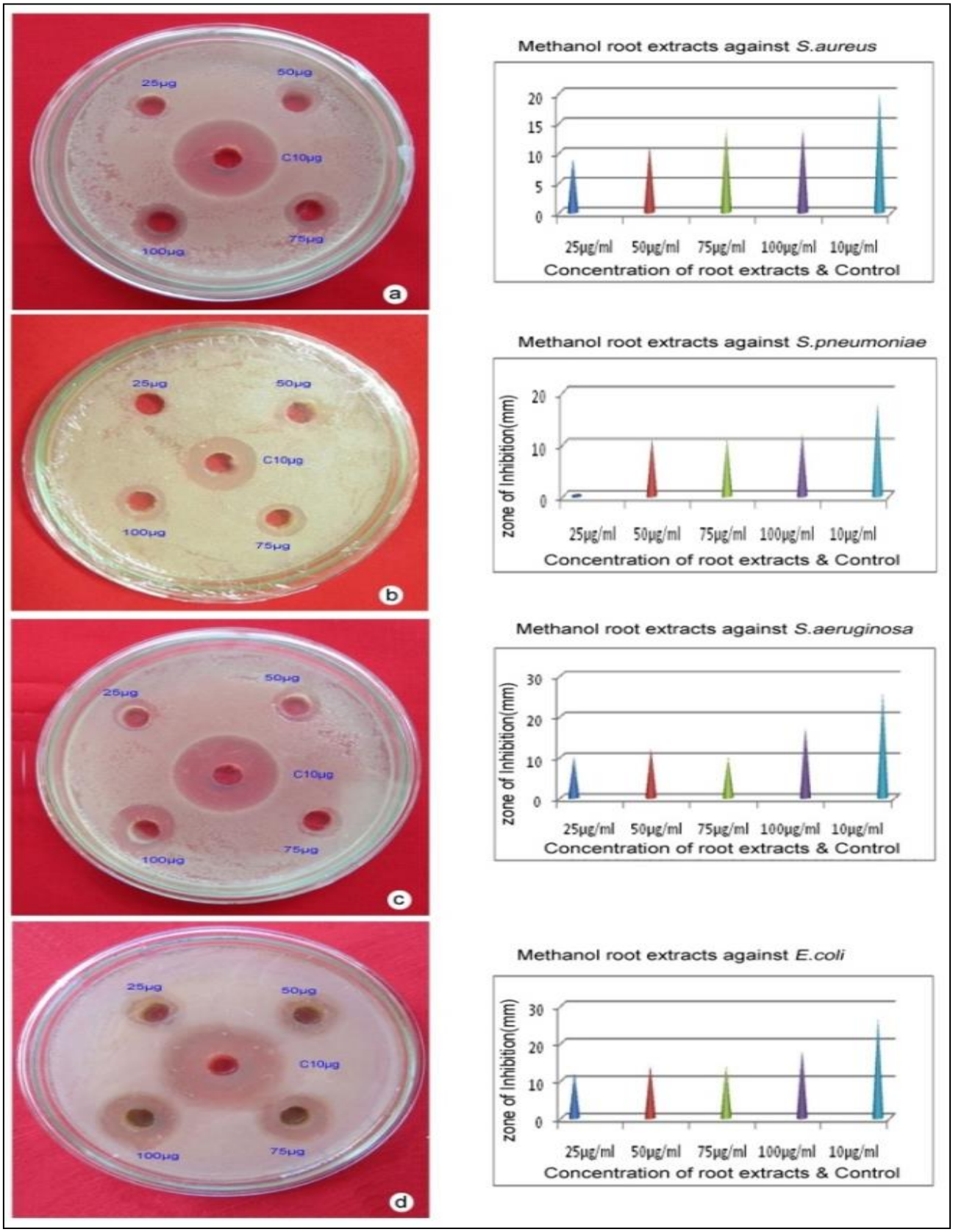

Fig 4: Bacterial activity of methanol root extract of *Momordica cymbalaria* Fenzl. (a) Inhibition zone of methanol root extract and streptomycin (control) against *Staphylococcus aureus*. (b) Inhibition zone of methanol root extract and streptomycin (control) against *Streptococcus pnemoniae*. (c) Inhibition zone of methanol root extract and streptomycin (control) against *Pseudomonas aerguniosa*. (d) Inhibition zone of methanol root extract and streptomycin (control) against *Escherichia coli*.

Conclusion

The result of the present study supported that, methanolic plant extracts of *Momordica cymbalaria*, possessed against different Gram positive and Gram negative bacteria, that can be used as antimicrobial agents in new drugs which are used in infectious diseases caused by microorganisms. The extracts were found to be effective against Gram negative (*E.coli* and *P. aeruginosa*) bacteria, when compared to Gram positive (*S. aureus*, *S. pneumonia*) bacteria. The phytochemicals present in the plant extracts may be a result for the antimicrobial activity.

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Compliance with ethical standards

Conflict of interest the authors declare that there are no conflicts of interest in this study.

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