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Investigation of Phytochemical Screening and Antibacterial Activity of Selected Species of *Salacia*

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Abstract: ***Salacia Linn belongs to the family Celastraceae which was formerly known as Hippocreataceae. It is the largest and most valuable genus possessing various Secondary Metabolites which have high medicinal properties. Hence, this study was focused on screening the presence of various phytochemicals such as steroids, terpenoids, tannins, flavonoids, phenols, carbohydrates, quinines, coumarins etc. Methanol was used as a Solvent system for the extraction process. Seven species of Salacia such as Salacia beddomei Gamble, Salacia chinensis L., Salacia fruticosa Heyne ex Lawson, Salacia gambleana Whiting & Kaul, Salacia macrosperma Wight, Salacia malabarica Gamble, and Salacia oblonga Wall, were selected for this study. The present study showed that steroid, flavonoid, saponins, tannins and alkaloid were found to be present in all species of Salacia whereas furan, quinone and phenol were absent in Salacia chinensis and Salacia oblonga respectively, terpenoid was absent in Salacia fruticosa, Salacia gambleana, carbohydrate was absent in Salacia macrosperma, Salacia malabarica and Salacia oblonga, coumarin was absent in Salacia beddomei, Salacia fruticosa, Salacia malabarica and acid was absent in Salacia chinensis, Salacia fruticosa and Salacia gambleana. Methanolic extract of selected species of Salacia was further subjected to antibacterial activity to find their inhibitory action against two bacterial species such as E-Coli (Gram +ve) and Bacillus cereus (Gram –ve). Various concentrations such as 250µg, 500 µg, 750 µg and 1000 µg were taken. All species of Salacia showed maximum inhibitory action against Bacillus cereus than E-Coli and the zone of inhibition was expressed in terms of Mean ± SD. Streptomycin was used as a standard positive control.***

Keywords: Salacia, Celastraceae, Phytochemicals, Methanol, E-Coli, Bacillus cereus, Zone of inhibition, Mean ± SD, Streptomycin.

I. INTRODUCTION

Herbal medicines are of great demand as they are rich in biological and medicinal properties, high safety margins and cost effective. They are used as a source of primary healthcare in both developed and developing countries. According to Angiosperm Phylogeny Group (2003) [1], *Salacia* belongs to the family Celastraceae which was formerly known as Hippocretaceae as most of their members are found to be nested within Celastraceae. It consists of about 200 species and found to be distributed in tropical areas such as India, Srilanka, Southern parts of China and South East Asian countries which was reported by Matsuda et al., (2005) [2].

Hasler et al., (1999) [3] explained Phytochemicals as a term derived from the greek word "Phyto" which means Plants. They are chemical compounds also called as Secondary Metabolites such as glycosides, flavonoids, tannins, alkaloids, steroids, phenols, anthraquinone, carbohydrates and cardiac glucosides. They are responsible for antioxidant activity, antimicrobial activity, stimulation of the immune system, enzyme activity, hormonal metabolism and cytotoxic study and they produced self defense to cure various diseases in mankind and was studied by Johnson et al., (2012) [4] and Mathai et al., (2000) [5] described that phytochemicals also contributes to colour, aroma and flavours in plants and protects plants from pollution, stress, drought, UV exposure and pathogenic attack.

Before the discovery of antibiotics, medicinal plants were used to treat various diseases of human beings. The pathogens are responsible to create a serious issue in the field of medicine as they have ability to transmit the resistance gene which was suggested by Davies and Davies (2010) [6].

Traditionally, herbal healers use medicinal plants as a anti-infective agents and plants based antimicrobials are found to possess enormous therapeutic values.

They are used in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials and was explained by Al-Bakri and Afifi (2007) [7].

Some of the species of *Salacia* are red listed as endangered species which was reported by Ravikumar and Ved (2000) [8]. Hence, the present study was focussed on the phytochemical analysis and antibacterial activity of selected *Salacia* species.

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II. MATERIALS AND METHOD

A. *Collection And Extraction Of Plant Materials*

Seven species of Salacia such as *S.beddomei, S.chinensis, S.fruticosa, S.gambleana, S.macrosperma, S.malabarica* and *S.oblonga*were collected from Wayanad district of Kerala. Leaves are collected and they are washed thoroughly and shade dried. They are coarsely powdered. 100g of powdered plant material was soaked in 300 ml of Methanol for 48hrs. The extract was filtered using Whattmann Filter paper and the filtrate was concentrated under reduced pressure in vacuum at 40°C for 25min using a rotary evaporator.

B. *Phytochemical Analysis*

The phytochemical screening of leaf extract of *S.beddomei, S.chinensis, S.fruticosa, S.gambleana, S.macrosperma, S.malabarica* and *S.oblonga* were assessed by the standard method as described by Sofowora (1993) [9], Trease et al., (1989) [10], Mace et al., (1963) [11], Kokata (1999) [12] and Harborne (1998) [13]. Methanolic extract of leaves of *Salacia* species were analysed and compared to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, alkaloids, terpenoids, glycosides, cardiac glycosides, coumarins and steroids.

C. *Antibacterial Assay*

1) *Test Organisms:* The present study includes two bacterial strains which includes one gram positive (G+) and gram negative (G-) bacteria such as *Escherichia coli* and *Bacillus cereus* respectively. The authenticated strains were collected from the division of Clinical Microbiology Pondicherry Centre for Biological Sciences, India.
2) *Growth and Maintenance of test organisms for Antibacterial Studies:* The bacterial isolates were sub cultured and established in Mueller Hinton Nutrient broth (Himedia, Bombay) at pH 7.4 and incubated at 37ºC in an incubator for 24 hours prior to study.
3) *Antibacterial Susceptibility Testing using Disc Diffusion Method:* Inoculum suspensions containing 10^8CFU/ml of bacteria equivalent to 0.5 McFarland standard were used for the study. The sterile Mueller Hinton Nutrient Agar (HiMedia, Bombay) was poured on to the sterile Petri plates and on solidification it is streaked with the suspensions of each test organisms and a lawn culture is established. Sterile discs (0.5 cm) obtained from HiMedia (Bombay) was loaded with 25µl of each extract dissolved independently in DMSO at a concentration of 1mg/ml as suggested by Romero (2005) [14]. The loaded discs were impregnated over the established lawn culture aseptically and the plates were incubated at 37ºC in an incubator for 24 hours. Zone of Inhibition (mm in diameter) were measured with three repeated experiments and the average of readings was taken as the antimicrobial activity against the test pathogen for the particular extract. Streptomycin (30µg/disc) were used as the positive control. Each assays were repeated thrice and the average values were recorded by Bauer et al., (1996) [15]

III. STATISTICAL ANALYSIS

The data was statistically analyzed by one-way ANNOVA using SPSS 17.0. The difference was considered significant when $p<0.005$.Triplicate assays were performed for each set of test conditions. All the values were expressed as Mean±SD (Standard Deviation). IC50 Value is also calculated for all test conditions.

IV. RESULTS AND DISCUSSION

A. *Phytochemical Screening*

The phytochemical screening of leaf extract of S.beddomei, S.chinensis, S.fruticosa, S.gambleana, S.macrosperma, S.malabarica and S.oblonga was carried out to study the presence or absence of various secondary metabolites as shown in Table 1. Methanol was used as a solvent system. The present study showed that steroid, flavonoid, saponins, tannins and alkaloid are present in all species of Salacia whereas furan, quinone and phenol are found to be absent in Salacia chinensis and Salacia oblonga respectively. Moreover terpenoid was absent in Salacia fruticosa and Salacia gambleana, carbohydrate was absent in Salacia macrosperma, Salacia malabarica and Salacia oblonga, coumarin was absent in Salacia beddomei, Salacia fruticosa, Salacia malabarica and acid was absent in Salacia chinensis, Salacia fruticosa, Salacia gambleana.

The phytochemicals can have complimentary and overlapping mechanism of action in the body including antioxidant effects modulation of detoxification enzymes, stimulation of the immune system and modulation of hormone metabolism. Secondary metabolites such as anthraquinones and derivatives of glycosides are found to contain cytotoxic, antimicrobial, antifungal, antitumor, antidiabetic, immune enhancing activity and was explained by Pandith et al., (2014) [16]. Also, in recent years phytochemicals have been used as a source of medicinal agents which was investigated by Krishnaraju et al., (1995) [17].

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Table 1 Phytochemical Analysis of Leaf Extract of *Salacia* Species

Phytochemicals	S.beddomei	S.chinensis	S.fruticosa	S.gambleana	S.macrosperma	S.malabarica	S.oblonga
Steroid	+	+	+	+	+	+	+
Terpenoid	+	+	-	-	+	+	+
Flavonoid	+	+	+	+	+	+	+
Furan	+	-	+	+	+	+	+
Carbohydrates	+	+	+	+	-	-	-
Coumarin	-	+	-	+	+	-	+
Quinone	+	-	+	+	+	+	+
Saponin	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+
Acid	+	-	-	-	+	+	+
Phenol	+	+	+	+	+	+	-
Alkaloid	+	+	+	+	+	+	+

B. Antibacterial Assay

Antibacterial activity was carried out for various species of *Salacia* such as *S.beddomei, S.chinensis, S.fruticosa, S.gambleana, S.macrosperma, S.malabarica* and *S.oblonga.* Methanolic leaf extract was used in various concentrations such as 250µg, 500 µg, 750 µg and 1000 µg in this study. Two bacterial strains which includes both gram positive (G+) and gram negative (G-) were used and they are *Escherichia coli* and *Bacillus cereus.* Zone of Inhibition was calculated and the values are interpreted in Mean ± SD as shown in Table 2.*Salacia* species showed maximum inhibitory action against *Bacillus cereus* than *Escherichia coli*and the zone of inhibition was expressed in terms of Mean ± SD. Streptomycin was used as a standard positive control which shows maximum inhibitory activity than all other species of *Salacia*.

Table 2 E-coli Inhibitory activity of selected Salacia species

Concentration (µg)	*SB* (Mean±SD)	*SC* (Mean±SD)	*SF* (Mean±SD)	*SG* (Mean±SD)	*SMac* (Mean±SD)	*SMal* (Mean±SD)	*SO* (Mean±SD)
250	6±0.7	7±0.6	6±0.8	7±0.7	7±0.7	6±0.8	6±0.7
500	8±0.8	8±0.7	8±0.7	9±0.6	8±0.6	7±0.7	8±0.6
750	9±0.7	10±0.7	9±0.6	10±0.8	10±0.7	9±0.8	9±0.7
1000	11±0.9	11±0.8	10±0.7	11±0.7	11±0.7	10±0.6	11±0.7
Standard	24±0.6	22±0.5	23±0.7	23±0.6	23±0.6	22±0.5	23±0.5

Table 3 Bacillus cereus Inhibitory activity of selected Salacia species

Concentration (µg)	*SB* (Mean±SD)	*SC* (Mean±SD)	*SF* (Mean±SD)	*SG* (Mean±SD)	*SMac* (Mean±SD)	*SMal* (Mean±SD)	*SO* (Mean±SD)
250	7±0.9	9±1.2	7±0.8	8±0.9	8±0.7	7±0.8	8±0.8
500	9±1.3	10±0.7	9±1.3	10±0.7	9±1.2	9±1.1	10±0.8
750	11±1.2	12±1.3	10±0.8	12±1.2	11±1.3	11±1.3	11±1.2
1000	13±1.4	14±1.4	13±1.3	13±1.4	13±1.4	14±1.3	13±1.3
Standard	23±0.7	24±0.6	24±0.7	24±0.6	23±0.8	24±0.7	24±0.6

(SB – Salacia beddomei, SC - Salacia chinensis, SF - Salacia fruticosa, SG - Salacia gambleana, S.Mac - Salacia macrosperma, S.Mal - Salacia malabarica and SO - Salacia oblonga).

According to Nair *et al* 2007, traditional medicines are used by 70% of the world population. Antibacterial property of many Indian Medicinal plants was studied based on folklore information and minimum reports are provided on inhibitory activity against certain pathogenic bacteria and fungi. The selection of plant products for antimicrobial activity has shown that the higher plants show an active source of novel antibiotic prototypes (Afolayan 1997).

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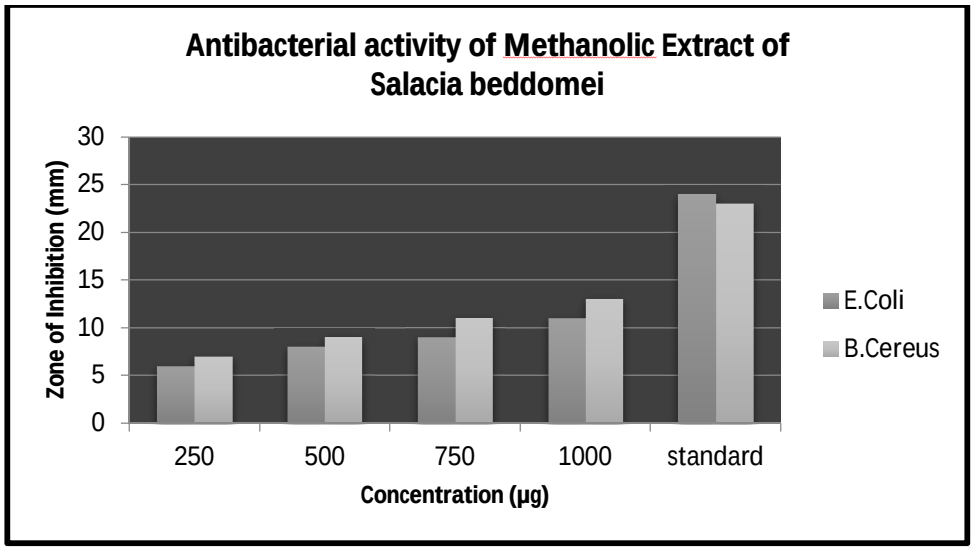
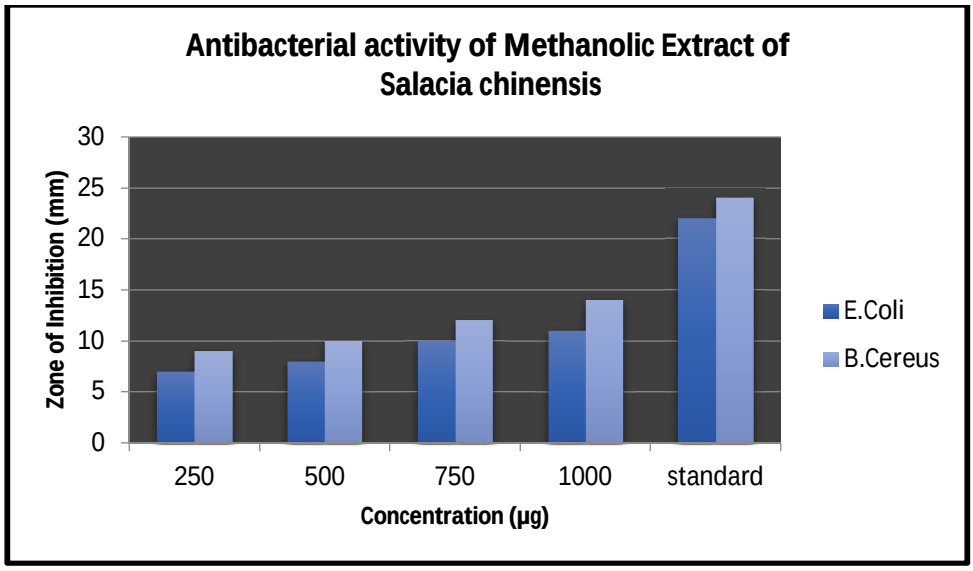
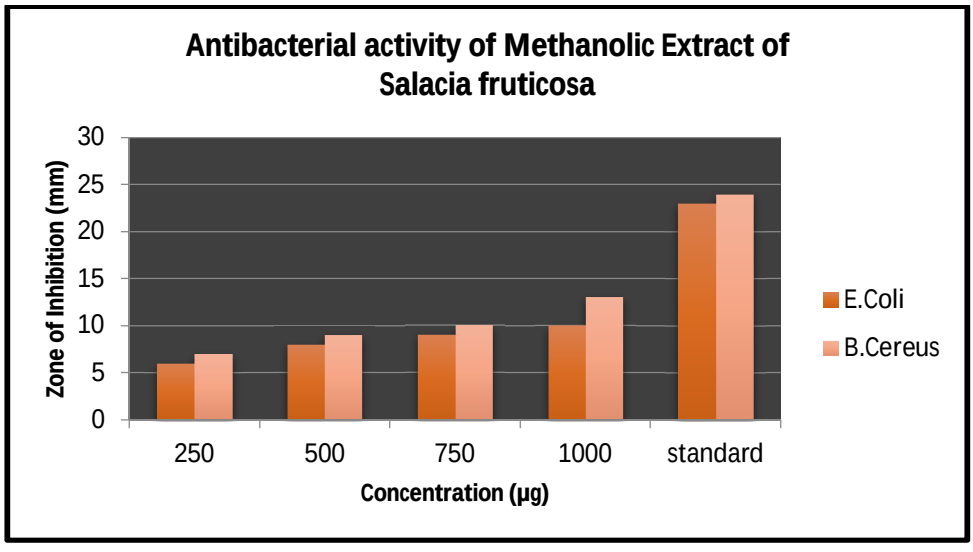
Figure 1 Antibacterial activity of Selected *Salacia* Species







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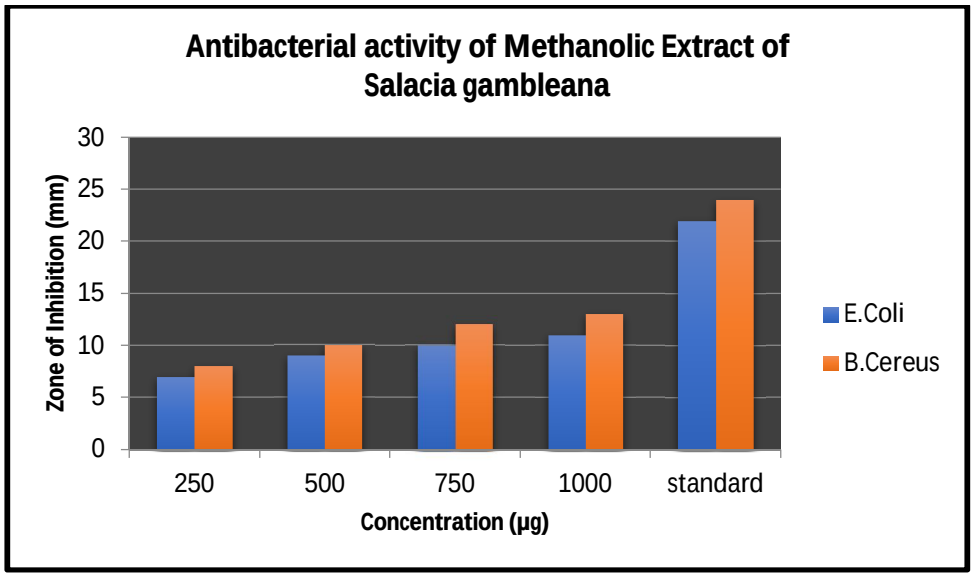

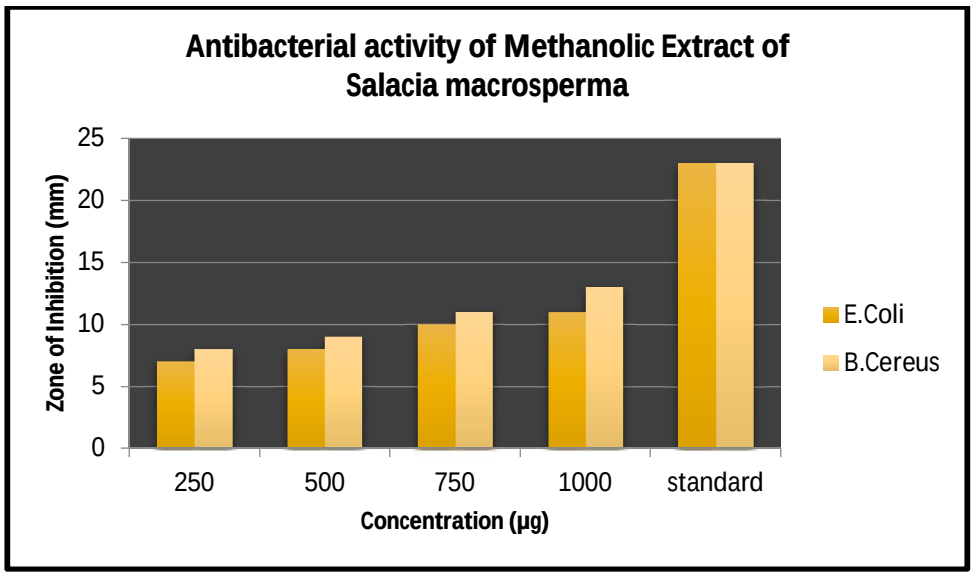

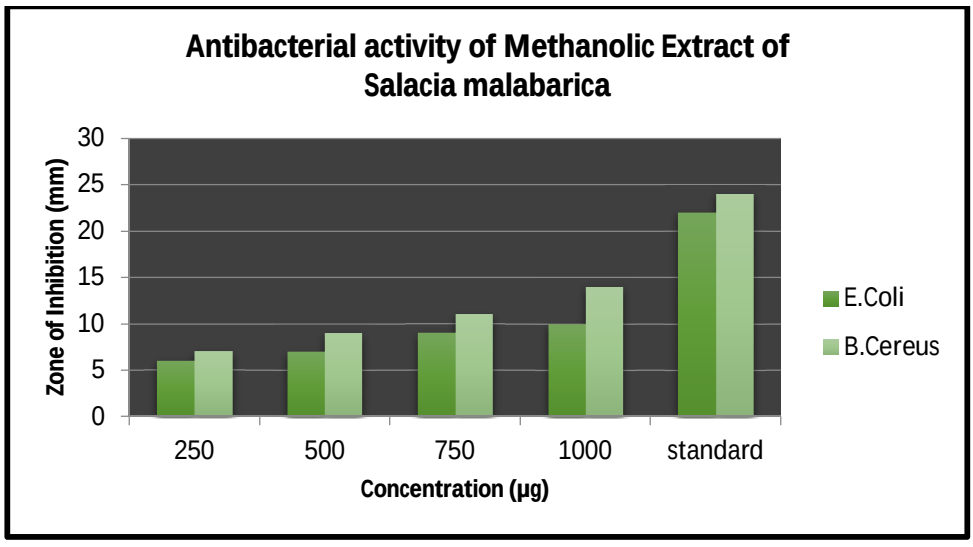

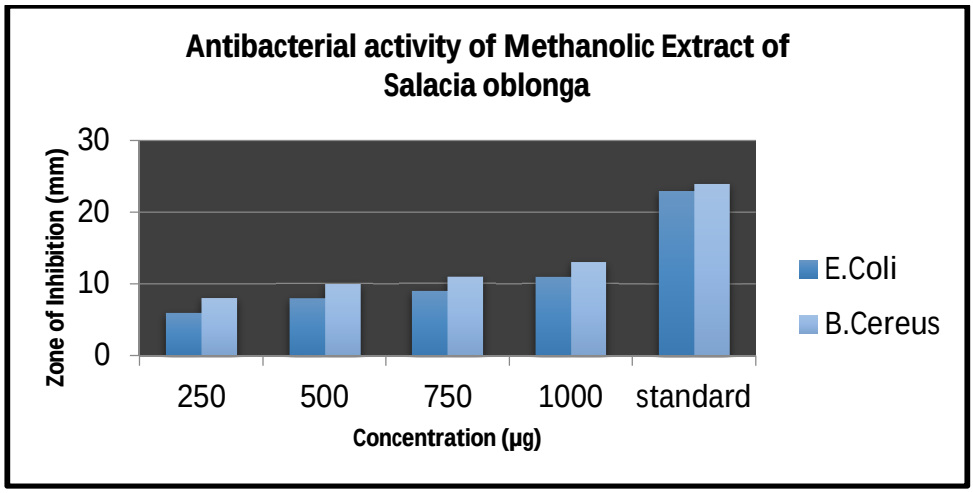

V. CONCLUSION

From the above results and observations it was concluded that secondary metabolites such steroid, flavonoid, saponins, tannins and alkaloid are present in all species of *Salacia* and all the studied species showed minimum inhibition against*Escherichia coli* than *Bacillus cereus.* The positive control Streptomycin showed higher inhibition than our study species of *Salacia*. Most of the species were red listed so this study helps to conserve them in future.

VI. ACKNOWLEDGEMENT

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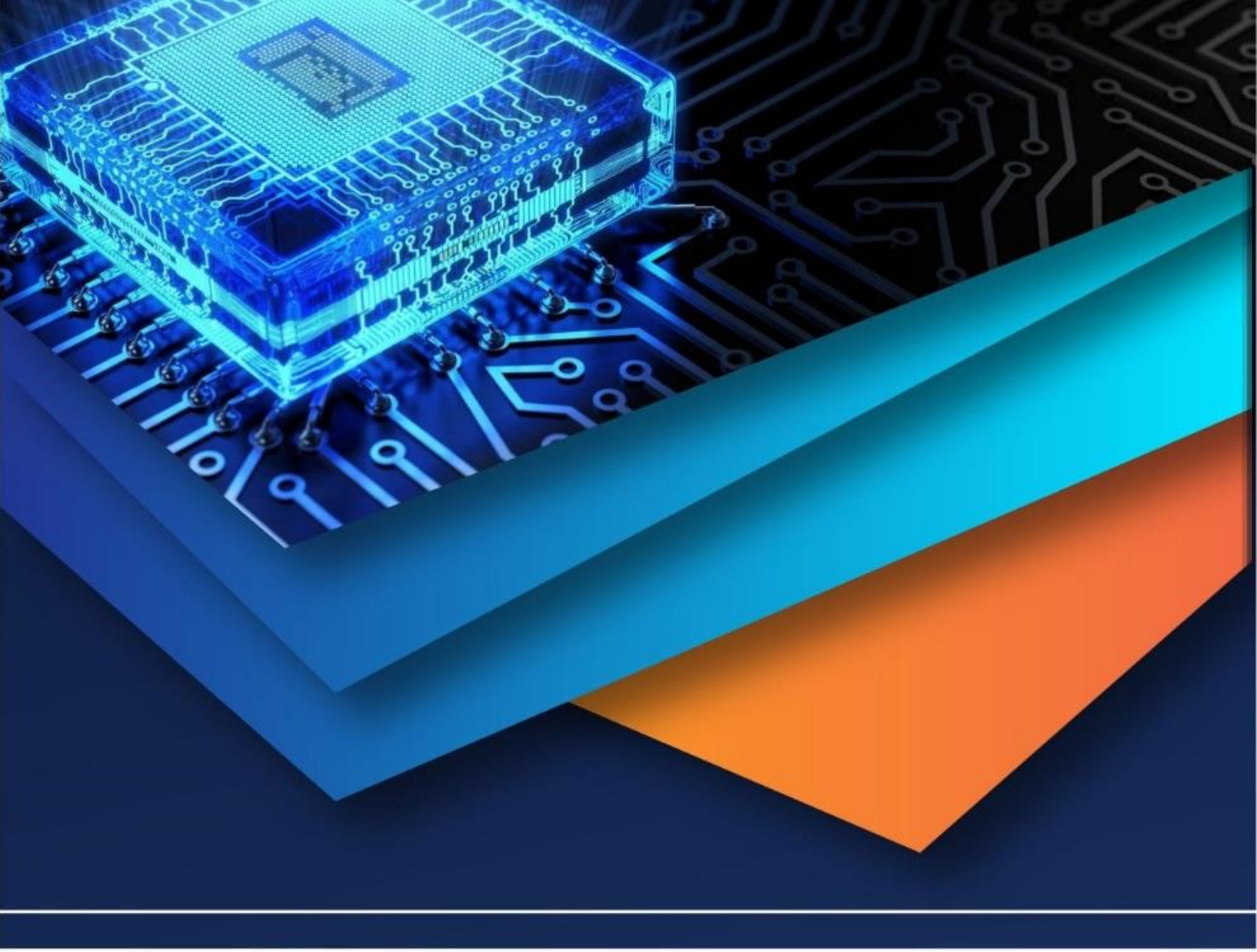

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