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Comparative Anti-microbial Activity of Different Extracts of Different Parts of *Butea monosperma*

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Abstract

Plants have served as a source of therapeutic therapies for millennia, and phytotherapy continues to play a crucial role in the primary health care of around 80% of the world's impoverished and developing nations. This study examined the antibacterial activity of *Butea monosperma* against *B. subtilis*, *S. aureus*, *S. typhi*, *L. acidophilus*, and *E. coli*. All *Butea monosperma* methanolic extracts exhibited the strongest antibacterial activity against *L. acidophilus*. Out of the four plant parts that were analyzed, the *Butea monosperma* stem extracts had the strongest antibacterial activity, followed by the flower and bark. *Leaf does not show any inhibition against* different bacterial strains. *Butea monosperma* has shown high antibacterial action against *B. subtilis*, *S. aureus*, *S. typhi*, and *E. coli*, in contrast to numerous co mmon drugs listed in Hexa g+7.

Keyword: *Butea*, antimicrobial, Drugs, Inhibition

Introduction

Medicinal plants possess many active compounds that may be advantageous for the formulation of medicinal drugs. The identification and isolation of phytochemical groups and/or individual chemical entities are essential for drug discovery, as these entities frequently function as standalone agents or as a collective group of phytocompounds (purified extracts) to attain the desired therapeutic effect. To assess their quality, the standardization of these plant parts must be conducted, encompassing a number of tests to ascertain the quality, amount, and purity of the phytocompounds. *Butea monosperma* is co mmonly known as Flame of forest, belongs to the family Fabaceae. It is locally called as palas, palash, mutthuga, kesudo, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino, Nourouc and is co mmon throughout India. Almost all the parts of the plant are being utilized since decades in medicine and for other purposes. These days herbal medicines are more trendy than modern medicine because of their effectiveness, easy availability, low cost and for being comparatively devoid of side effects. Plants have been a source of medicinal treatments for eons, and phytotherapy continue to play a fundamental role in primary health care of about 80% of the world's underdeveloped and developing countries. The trend of using complementary and alternative medicines (CAM) has expanded in recent years both in the developing and developed countries. Due to limited research, findings on proving safety and efficacy of CAM are scarce. Hence, time demands pharmacological profiling of such drugs in order to identify the ones that are truly effective.

Materials and methods

Bacterial strains

Five organisms were used for the study: *Salmonella typhimurium* (MTCC no 3224), *Lactobacillus acidophilus* (MTCC no 10307), *Staphylococcus aureus* (MTCC no 3160), *Escherichia coli* (MTCC no 1610), and *Bacillus subtilis* (MTCC no 441). To assess vitality, each isolate was resurrected using nutritive agar-medium and Sabouraud's dextrose-agar (SDA) media. The stock cultures were maintained on nutritious agar medium (Hi Media, Mumbai, India) and potato-dextrose agar medium. To screen for bacteria, they were grown for 24 hours at 37 °C. After that, they were stored in a refrigerator at 4°C until sensitivity testing was necessary.

Making a *Butea* Extract:

After being bought from the neighborhood market, the *Butea* plant parts were cleaned and, if required, descaled before being rinsed in sterile distilled water. One gram of *Butea* pulp was dissolved in five milliliters of a 50% aqueous ethanol solvent in a 250 mL Erlenmeyer flask to create the herbal extract. The best solvent for removing the physiologically active phytochemicals from the *Butea* pulp was found to be this 50% aqueous ethanol solution. Water, ethanol, isopropanol, petroleum ether, and chloroform were among the other solvents that were tested. After sealing the flasks with aluminum foil and cotton plugs, the *Butea* pulp was left to soak in the 50% aqueous ethanol for 48 hours at room temperature, shaking them occasionally. The mixture was soaked, centrifuged for 20 minutes at 3500 g, and then filtered through Whatman filter paper No. 1. The pellet was disposed of, and a rotating vacuum evaporator was used to collect and concentrate the supernatant at a lower pressure until a semisolid residue was obtained. To create a solid powder, this residue was subsequently dried in a crucible at a regulated temperature of 45 ºC. Until 500 mg of powder were collected, the extraction procedure was repeated. Prior to being sterilized using a 0.45-micron membrane filter, the powder was weighed, reconstituted in dimethyl sulfoxide (DMSO), and sieved through fine mesh cloth. For antimicrobial sensitivity testing, this extract, which represented a 100% concentration, was kept in a refrigerator at 4 ºC.

Anti-bacterial activity testing using agar well-method (cup-plate method)

The positive control was sodium propionate, a co mmon food preservative, and the negative control was sterile DMSO. After allowing the extract to diffuse for at least an hour, the plates were incubated for 24 hours at 37ºC. To the closest millimeter, the zones of inhibition were measured (NCCLS, 1999). Every experiment was carried out three times. Sterile DMSO served as the negative control, and sodium propionate, a co mmon food preservative, served as the positive control. The plates were incubated for 24 hours at 37ºC after the extract had been allowed to diffuse for at least an hour. The zones of inhibition were measured to the nearest millimeter (NCCLS, 1999). All experiments were conducted three times.

Results and Discussions

Alkaloids, tannins, saponins, glycosides, flavonoids, anthraquinones, reducing sugars, terpenoids, and phenols were among the substances found in *Tamarindus indica* leaves and fruit extracts after phytochemical screening. The antibacterial qualities of the plant are facilitated by these compounds. In particular, saponins are recognized for their antibacterial qualities (Gonzalez–Lamothe *et al*., 2009; Cowan, 1999), whereas flavonoids have shown anti-infla mmatory, anti-hepatotoxic, and antimicrobial activities. Additionally, tannins have antibacterial properties and promote wound healing. Alkaloids are a broad class of nitrogenous compounds that are present in both leaves and fruits. They are employed in a variety of medical applications, including as anesthetics, central nervous system stimulants, and anticancer therapies. Alkaloids have an impact on cell division and metabolism, which may account for their antibacterial properties.

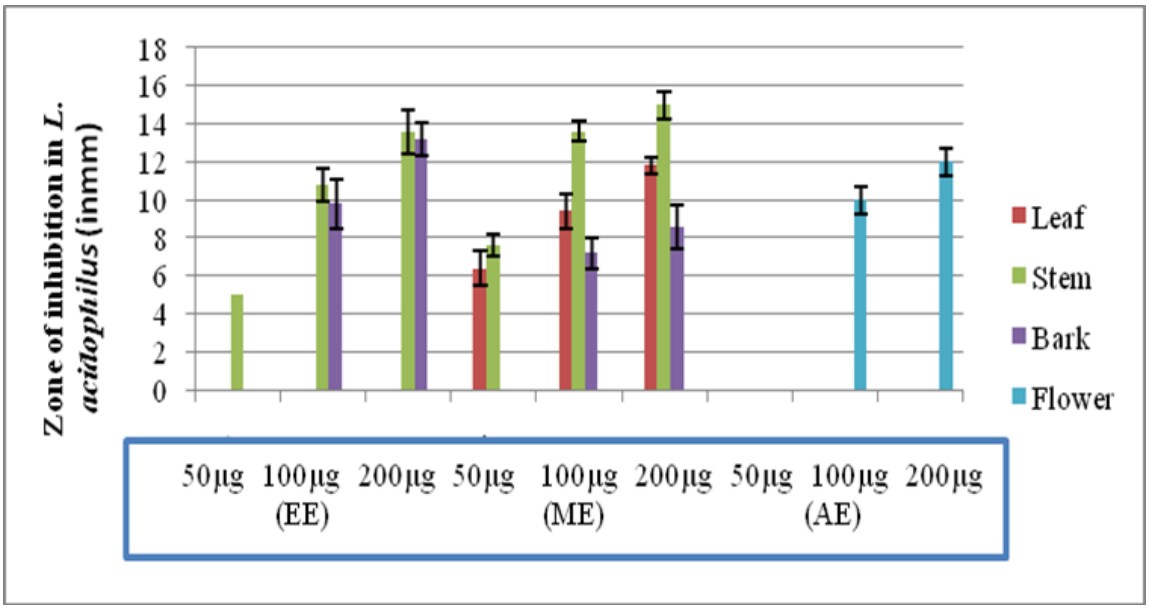
Phytochemical examinations of different parts of the *Butea monosperma* plant, including its flowers, gum, seeds, resin, sap, bark, stem, and leaves, have been carried out using various solvent extracts such as ethanol, methanol, water, petroleum ether, chloroform, and acetone (Prashanth *et al*., 2001; Sindhia and Bairwa, 2010; Ajay and Neetu, 2011; Hajare *et al*., 2013). Prashanth *et al*. (2001) performed an initial qualitative analysis of phytochemicals in different aerial parts of *Butea monosperma*, detecting tannins, carbohydrates, terpenoids, glycosides, and alkaloids in the aqueous extracts, along with serroils in the ethanolic extracts. Sindhia and Bairwa (2010) provided a review of the chemical constituents found in various parts of the *Butea* plant. Prashanth *et al*. (2012) documented the presence of sterols, triterenes, triterpenes, glycosides, flavonoids, and proteins in the petroleum ether extract of the *Butea* roots. Dhale *et al*. (2010) discovered tannins, crude proteins, and reducing sugars in leaf extracts. Sahu *et al*. (2013) identified saponins and tannins in the ethanol, methanol, and aqueous extracts of leaves, in addition to alkaloids, carbohydrates, and glycosides in the ethanol and water extracts. Our research is consistent with these observations, though we identified some variations. Notably, glycosides were not detected in the water extract of the leaves, while both anthraquinones and flavonoids were found in the ethanolic and aqueous leaf extracts. Furthermore, the methanolic leaf extract from our investigation included proteins and sterols.

Table 1: Qualitative profile of phytochemicals in *Butea monosperma* leaf, stem, bark and flower ethanolic, methanolic and aqueous extracts.

S. No.	Phytochemical Name	Leaf			Stem			Bark			Flower		
		EE	ME	AE	EE	ME	AE	EE	ME	AE	EE	ME	AE
1	Alkaloids	+	-	+	+	+	-	+	-	-	+	-	-
2	Flavonoids	+	+	+	+	+	+	-	+	+	-	+	+
3	Tannins	+	+	+	+	-	-	+	+	+	-	-	-
4	Saponins	+	+	+	+	+	+	+	-	-	+	+	-
5	Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
6	Phytosterols	-	+	-	-	-	-	-	-	-	+	+	-
7	Glycosides	+	+	-	+	-	-	+	-	-	+	+	+
8	Protein	-	+	-	+	-	-	+	-	-	-	-	-
9	Reducing sugars	-	-	-	-	+	-	+	+	-	+	+	+
10	Anthroquinones	+	-	+	+	+	-	+	-	-	+	-	-

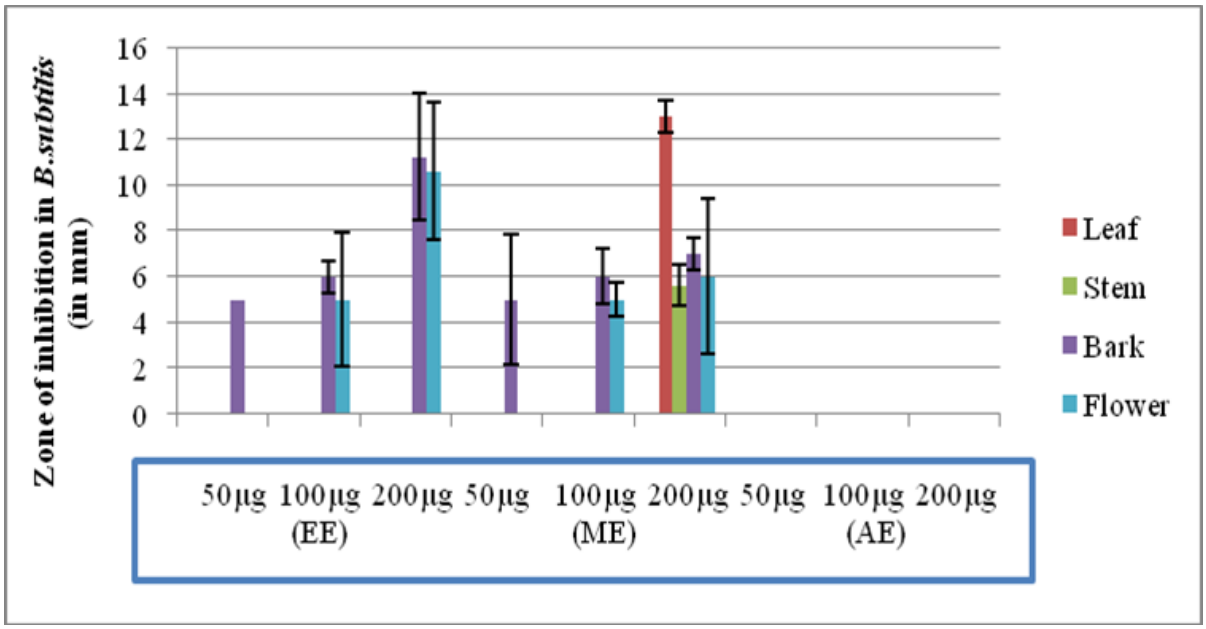
Antibacterial activity of *B. monosperma* plant parts leaf, stem, bark and flower against *L. acidophilus* strain

In *Butea*, methanol extracts of leaves, stems, and bark also showed antibacterial activity against *L. acidophilus*. However, ethanolic (stem and bark) and aqueous (flower) extracts showed limited activity. The maximum inhibition observed was 15.0±0.71 mm for 200 µg methanolic extract, followed by stem extract (100 µg methanol and ethanol) and cortex extract (200 µg ethanol). Increasing concentrations of methanolic extracts of stems and bark gave results comparable to those of standard antibiotics. Our results show that methanol extracts *butea* stem are less effective against *L. acidophilus* compared to standard antibiotics.



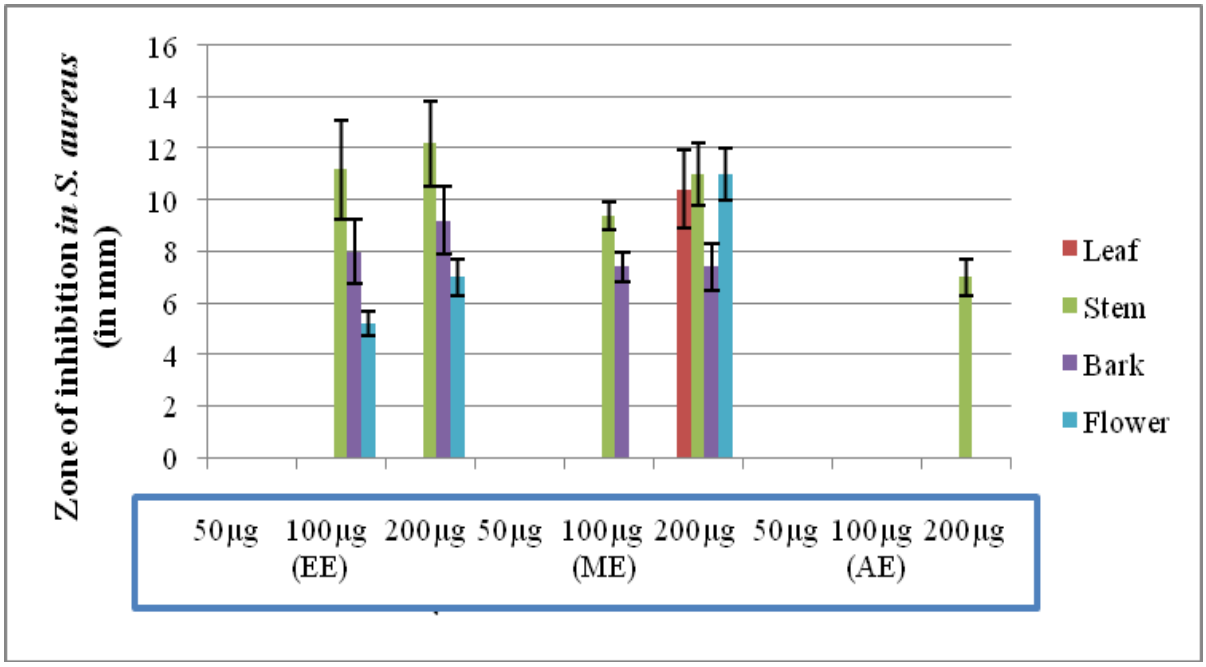
Antibacterial activity of *B. monosperma* plant parts leaf, stem, bark and flower against *B. subtilis* strain

Our study showed that *B. subtilis* was sensitive only to six *butea* extracts. Methanolic extracts from the leaf, stem, bark, and flower, as well as ethanolic extracts from the bark and flower, demonstrated antimicrobial activity against *B. subtilis*. In contrast, the aqueous extract showed no antibacterial activity against *Bacillus subtilis*. Methanolic extract of leaves showed the highest zone of inhibition of 13.0±0.71 mm at 200 µg, which was higher than OX antibiotic activity (13.0±1.87 mm).



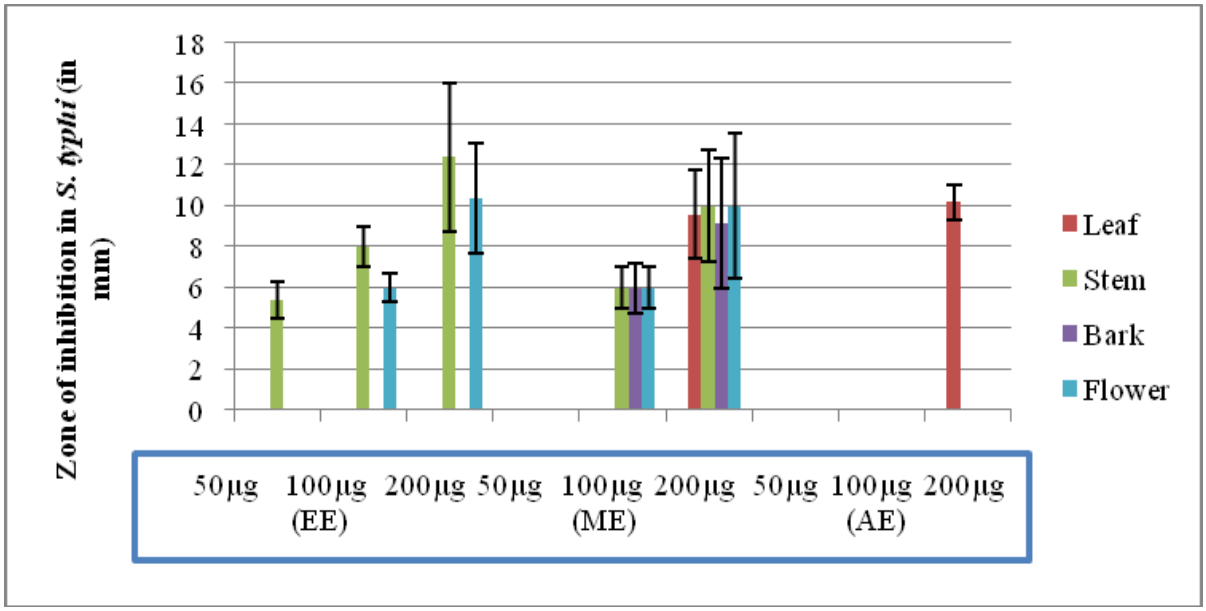
Antibacterial activity of *B. monosperma* plant parts leaf, stem, bark and flower against *S. aureus* strain

Leaf does not show any activity in ethanolic and aqueous extracts while in aqueous extract flower and bark also does not exhibit any activity. However in methanolic and ethanolic extracts stem bark and flower exhibited antibacterial activity.



Antibacterial Activity against *S. typhi*

Ethanolic extracts showed varying degrees of sensitivity, with stem and flower extracts demonstrating notable antimicrobial activity. At 50, 100, and 200µg concentrations, stem extracts produced inhibition zones of 5.4±0.89 mm, 8.0±1.0 mm, and 12.4±3.65 mm, respectively. Flower extracts showed inhibition zones of 6.0±1.0 mm at 100µg and 10.4±2.7 mm at 200µg. In contrast, ethanolic extracts of leaf and bark did not exhibit any antimicrobial activity against *S. typhi*.

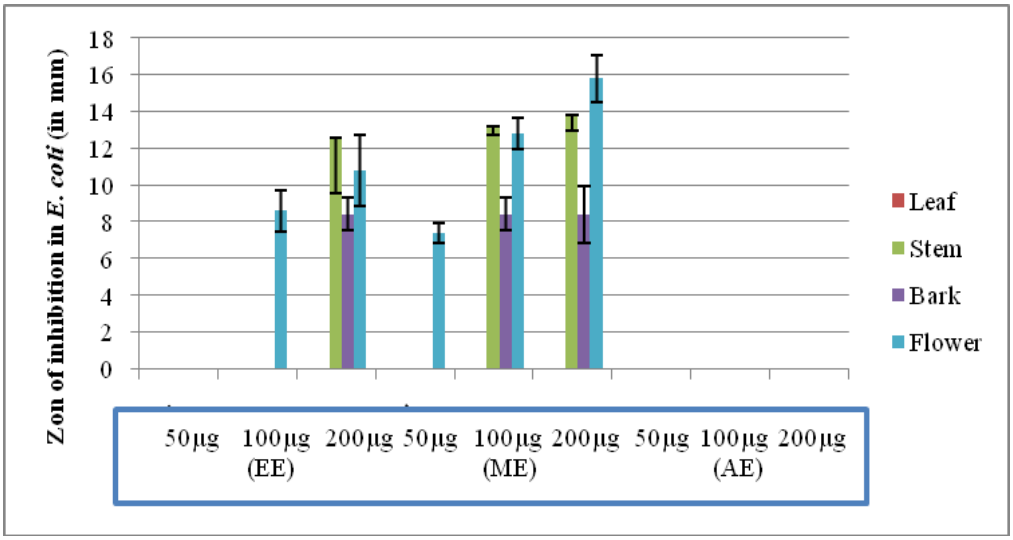


Methanolic extracts demonstrated low to high sensitivity. At 100µg, methanolic extracts of stem, bark, and flower each produced an inhibition zone of approximately 6.0 mm. At 200µg, all methanolic extracts (leaf, stem, bark, and flower) displayed high sensitivity, with inhibition zones significantly larger than at lower concentrations. Meanwhile in aqueous extracts only leaf exhibited the antibacterial activity against *S. typhi*.

Antibacterial activity of *B. monosperma* plant parts leaf, stem, bark and flower against *E. coli* strain

The ethanolic extract from the leaf showed no inhibitory effect on *E. coli* growth. However, the stem and bark extracts demonstrated inhibition, with the stem extract achieving up to 12.6±3.05 mm and the bark extract up to 8.4±0.89 mm zone of inhibition at 200µg concentration. Additionally, the flower extract inhibited *E. coli* growth at concentrations of 100 and 200µg, with inhibition zones of 8.6±1.14 mm and 10.8±1.92 mm, respectively.

The methanolic leaf extract also showed no activity against *E. coli*. The stem methanolic extract displayed very high sensitivity (++++) with inhibition zones of 13.2±0.45 mm and 13.8±0.84 mm at 100 and 200µg concentrations. The bark methanolic extract had medium sensitivity (++) with a zone of inhibition of about 8.4 mm. The flower methanolic extract effectively inhibited *E. coli* growth at 50, 100, and 200µg concentrations, with inhibition zones of 7.4±0.55 mm, 12.8±0.84 mm, and 15.8±1.3 mm, respectively. No aqueous extracts showed antimicrobial activity against *E. coli*.



In vitro studies have previously reported antibacterial properties of *Butea* extracts from different parts including leaves (Sahu and Padhy 2013), bark (Gurav *et al*., 2008), roots (Tiwari, 2012) and pods (Jayshree, 2015). Sahu and Padhy (2013) found that among various soluble extracts, aqueous extracts were more effective than others. Jayshree (2015) reported potent activity of ethanol extract of *butea* pods against *Salmonella typhi*, whereas Gaurav (2008) observed minimal inhibition by petroleum ether and ethanol bark extracts. In our study, *B. monosperma* was found to be associated with *S. typhi.*

Previous *in vitro* studies on the sensitivity of *E. coli* to *Butea* plant extracts have used various solvents including water, ethanol, methanol, petroleum ether, acetone, ether and chloroform (Garav, 2008; Mehta, 2010; Dhel, 2010; Rajput *et al*., , 2011; Malpani *et al*., 2012; Sahu and Padhy , 2013; Jayshree (2015) found that *E. coli* was more susceptible to ethanol extract of pods at a concentration of 31.25 mg/ml. Singh (2011) announced the sensitivity of NTCC *E. coli* shares to the cortex of *Butea* Ethhanol, but Rmanjaneuulu *et al*., (2011) observed the leaf water extraction activity to *E. coli*. Malpani *et al*., (2012) reported that *E. coli* was susceptible to all aqueous extracts of *Butea* stems, bark, and flowers. However, in our study, all the aqueous extracts, including the leaf extract, failed to inhibit the growth of *E. coli*. Methanol extract of flowers at a concentration of 100 µg showed the highest antibacterial activity against *E. coli* compared to other *Butea* extracts.

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