



## Comparative Evaluation of Anti-Microbial Activity of Between Combination of Extract of *Pogamia pinnata* with Caffeine and *Pogamia pinnata*: *In Vitro* Approach

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### ABSTRACT:

The antimicrobial activity of a novel combination of plant-based extracts of *Pogamia pinnata* and caffeine, as determined by *in vitro* studies, is investigated in this study. Since antimicrobial resistance is becoming a major public health concern, there is a growing interest in investigating alternative antimicrobial agents. *Pogamia pinnata* have long been known to have antimicrobial properties, and caffeine has emerged as a potential enhancer of antimicrobial efficacy. However, the synergistic antimicrobial effects of combining *Pogamia pinnata* with caffeine are still not well understood. In this study, we carried out a comparative evaluation of anti microbial properties *in vitro* experiments, between extract of *Pogamia pinnata* with caffeine and extract of *Pogamia pinnata* (*in vitro*). In this study we are investigated that *Pogamia pinnata* (10% EESPP) has antimicrobial activity against *S. aureus* (gram +ve), *E. coli* (gram -ve), *S. Typhi* (gram -ve), and *P. aeruginosa* (gram -ve). However, when caffeine is combined with *Pogamia pinnata* 10% EESPP (50 µl), the combination exhibits greater inhibitory activity against microorganisms than *Pogamia pinnata* 10% EESPP (50 µl) alone. The study shows caffeine increases antimicrobial activity of the ethanolic extract of *Pogamia pinnata*.

**Keywords :** Anti-microbial Activity, Microbial Activity, Herbal Plants, Caffeine, EESPP.

### INTRODUCTION :

A variety of pharmacological activities, including antidiabetic, antihyperglycemic, antiliperoxidative, depressant, antiinflammatory, analgesic, antifilarial, antioxidant, antihyperammonemic, antiviral, antifungal, antibacterial, and antimicrobial activity, have been reported for *Pogamia pinnata* Linn. (Family: Fabaceae) [1].

Antihyperglycemic, antihyperliperoxidative, depressive, antiinflammatory, analgesic, antifilarial, antioxidant, antihyperammonemic, antiviral, antifungal, antibacterial, and antimicrobial properties are also present in this plant. *Pogamia pinnata* seeds and seed oil are traditionally used to treat wounds in the Unani and Ayurvedic systems [2].

There have been reports of *P. pinnata*'s efficacy as a source of biomedicines [3], particularly as antibacterial and therapeutic compounds. The ethanolic stem extract's antibacterial properties against four bacterial species—*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*—were also examined in this plant [4].

Caffeine (1, 3, 7 – trimethylxanthine) is a member of methylxanthines, a class of compounds that is widely distributed in nature. Caffeine is regularly consumed from dietary sources including coffee, tea, cola beverages and chocolate [5, 6].

Additionally, concurrent use of caffeine with antibiotics has been shown to increase the antibacterial activity of amoxicillin, decrease the bactericidal activity of benzylpenicillin, and have almost no effect on ampicillin's antibacterial activity [7]. It has been demonstrated that caffeine increases the anti-*Staphylococcus aureus* inhibitory effect of penicillin G and tetracycline [8]. It has been reported that caffeine increases the anti-*Staphylococcus aureus* inhibitory effect of penicillin and tetracycline additionally showed increase effect of furazolidone against vibrios[9].



It is worthwhile to conduct a comparative assessment of the antimicrobial activity between ethanolic extract of stem bark of *Pongamia pinnata* with caffeine (EESPP + caffeine) and ethanolic extract of stem bark of *Pongamia pinnata* (EESPP) because no previous work has been published on this work.

## 1 MATERIALS AND METHODS

### 1.1 Sample collection and Extract preparation

In April and May, fragments of *Pongamia pinnata* stem bark were gathered from the hilly region of Amravati in the Indian state of Maharashtra. After being shade-dried, the stem bark of *Pongamia pinnata* was ground into a powder (Mesh size-16) in a grinder [10]. The 500 g of powdered bark was macerated in 2.5 L of 99.9% alcohol for seven days at room temperature before being filtered. A tray drier was used to dry the filtrate at 40 °C (yield: 3.679% w/w)[10]. Standard deviation and standard error were used to statistically analyze the data.

### 1.2 Preparation of Extract:

The drug extract was prepared by taking 10 % EESPP (4.2 g) in 50ml ethanol.

### 1.3 Sampling of microorganism:

We collected different sample of microorganisms in form of infected blood aseptically brought from pathogen laboratory.

### 1.4 Collection of Caffeine:

We used the caffeine drug collected from nearby supplement store. Manufactured and marketed by Novus Life Science Pvt. Ltd.

### 1.5 Preparation of caffeine:

1.2gm caffeine dissolves in 50ml ethanol.

### 1.6 Positive control: Streptomycin

### 1.7 Culture media:

The media use for the study was Nutrient Agar Medium (NAM) used for the microbial growth.

## 2 Agar well diffusion assay

Antimicrobial activity was tested using the well diffusion technique. We allowed the diffusion of the substances, each sterilized Petri plate was pre-seeded with 15 ml of the appropriate growth agar medium and 80 µl of microbial culture and lawn. The lawn was then prepared using the spread plate method and allowed to dry for 30 minutes before wells were made using a sterile cork borer. The wells were (A) 10 percent EESPP (50 µl), (B) 10 percent EESPP + caffeine (50 µl), and finally (C) Positive control (Streptomycin) (50 µl). Comparative analysis of the antimicrobial activity of a plant extract and caffeine combination (in vitro). Calculate the zones of inhibition based on the data that was obtained [11, 12]. The media use for the study was Nutrient Agar Medium (NAM) used for the microbial growth. There were two petri dishes Petri dish 1 and Petri dish 2 In which had add 4 different (infected human blood sample) microorganism as follow respectively.

- *Staphylococcus aureus* (gram +ve),
- *Escherichia coli* (gram -ve),
- *Salmonella Typhi* (gram -ve),
- *Pseudomonas aeruginosa* (gram -ve),

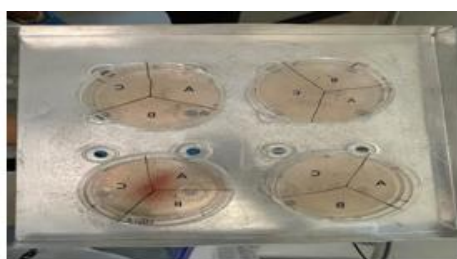
### 3 Statistical analysis:

This Experiment was performed in triplicate in an independent manner. The data were expressed as mean and standard error of three replicates and values were analysed statistically (Figure 1).

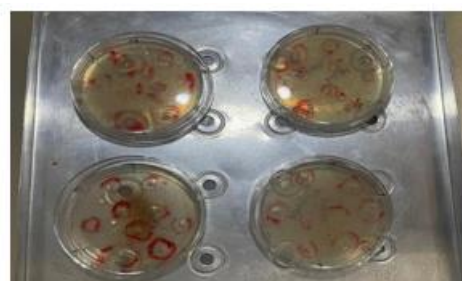
### 4 Result:

Data are means of three replicates (n=3)±standard error.

Microorganisms	10% EESPP	10% EESPP +Caffeine	Streptomycin
<i>Staphylococcus aureus</i>	39.2 ±0.61	42 ±0.01	90 ±0.46
<i>Escherichia coli</i>	15.3 ±0.21	18.2 ±0.64	82 ±0.35
<i>Salmonella Typhi</i>	37 ±0.11	38 ±0.31	85 ±0.15
<i>Pseudomonas aeruginosa</i>	31.4 ±0.24	32 ±0.53	79 ±0.35



**Fig no.1. Petri Dishes at 1<sup>ST</sup> day  
Zone of Inhibition**



**Fig no.2. Petri Dishes after 24 hr Containing**

### 5 Discussion:

Most of food poisoning reports are associated with bacterial contamination especially members of Gram negative bacteria like *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* [13, 14]. Other Gram positive bacteria including *Staphylococcus aureus* and *Bacillus cereus* have been also identified as the causal agents of food borne diseases or food spoilage [15]. Caffeine is CNS stimulant but also own antimicrobial activity caffeine also enhanced inhibitory effect of some antimicrobial agent like penicillin and tetracycline. *Pongamia Pinnata* Linn. (Family: Fabaceae) are known to possess various pharmacological activity which are published like antidiabetic, antihyperglycaemic, antiliperoxidative, depressant, antiinflammatory, analgesic, antifilarial, antioxidant, antihyperammonemic, antiviral, antifungal activity. Zone of inhibition due to 10 percent EESPP+caffeine is large as compare to Zone of inhibition due 10 percent EESPP. Zone of inhibition due to 10 percent EESPP+caffeine is near to Zone of inhibition due streptomycin (+v control). Here, as shown in above observation, *Pogamia Pinnata* stem bark have antimicrobial activity against *s. aureus* (gram +ve), *E.coli*(gram-ve), *S.Typhi*(gram -ve), *P.aeruginosa*(gram -ve), but in combination of caffeine with 10 percent EESPP (50 µl), have more inhibitory activity against microorganism as compare to 10 percent EESPP (50 µl). In presence of caffeine, antimicrobial property of ethanolic extract of stem bark *Pongamia Pinnata* is enhanced.

### 6 Conclusion

Further study is require to evaluate the bioactive compounds present in various organic extracts *Pongamia pinnata* are to be used for drug or food preservation, issues of safety and toxicity will always need to be addressed. Further study is require to evaluate mechanism of action of caffeine by which it enhance antimicrobial effect of *Pongamia pinnata*.

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Conflict of Interest Statement: All authors have nothing else to disclose.

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