

ANTIMICROBIAL AND CYTOTOXIC EFFECT OF *CAMELLIA SINENSIS* AND *ACACIA NILOTICA* HERBAL FORMULATION

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ABSTRACT :

INTRODUCTION: *Camellia sinensis* (Green tea) and their polyphenols, have attracted great attention over the years as possible nutraceuticals, because of their multiple bioactivities, especially antioxidant and anti-inflammatory activity, which could be exploited in several diseases, including such skin ailments. *Acacia nilotica* (Babool) has more medicinal uses and it has potent antioxidant activity. It provides nutrients and therapeutic ingredients for treatment of many diseases. The aim of the study is to evaluate the combined action of *Camellia sinensis* (Green tea) and *Acacia nilotica* (Babool) herbal formulation whether it has better antimicrobial and cytotoxic effect

MATERIALS AND METHODS: 1g of green tea and babul powder was weighed aseptically and then dissolved in 100ml of distilled water. Then the solution is boiled for about 5 minutes at a temperature of about 60-80 degree celsius and then allowed to cool down followed by filtration of extract and then copper nanoparticles were synthesized and antimicrobial and cytotoxic activity was done.

RESULTS: Antimicrobial activity was noted in different pathogens like *E. faecalis*, *C.albicans*, *S.mutans* and *S. aureus*. It was seen that there was an increased zone of inhibition seen in the control group of all pathogens. It has been observed that no change was observed in live nauplius in day 1 and after that differs in each concentration it was effective in 5µl than other concentrations.

And there was no change observed in the control group. From the study it can be clearly seen that a concentration dependent Antimicrobial activity and cytotoxic effect seen which is evident in herbal formulation.

CONCLUSION :It has been concluded that the green tea and babul herbal formulations have better antimicrobial and cytotoxic activity.

KEYWORDS: Anti-inflammatory agent, *Camellia sinensis*, *Acacia nilotica*, Herbal extract.

INTRODUCTION:

Green tea and their polyphenols, have attracted great attention over the years as possible nutraceuticals, because of their multiple bioactivities, especially antioxidant and anti-inflammatory activity, which could be exploited in several diseases, including such skin ailments (1). Moreover it has many uses, one of which is helping overweight people to lose weight and to maintain weight loss. And it was believed to be able to increase a person's energy output, green tea weight loss preparations are extracts of green tea that contain a higher concentration of ingredients such as catechins and caffeine than the typical green tea beverage prepared from a tea bag and boiling water (2). The leaf extract of *Acacia nilotica* (babool) is rich in different types of plant secondary metabolites such as flavonoids, tannins, triterpenoids and saponines. Various parts of babool such as leaves, bark, roots, and fruits are widely used as medicine for the treatment of bronchitis, asthma, meningitis, hypoglycemia, pneumonia and sore throat (3).

Antioxidant potential of the plant extract depends on the presence of phenolic compounds and flavonoids. From the past research, it was already proved that the phenolic content is directly responsible for reduction of oxidative stress. Antioxidants are the substances which significantly destroy the free radicals—reactive oxygen species responsible for degenerative diseases. Major plant antioxidants are secondary metabolites wherein phenyl-propanoid metabolism includes phenolics, coumarins, tannins, chalcone and flavonoid (4). Two possible mechanisms of actions have been postulated; hydrogen or electron donating ability confirmed by reducing power and DPPH radical scavenging assays and direct free radical scavenging property confirmed by lipid peroxidation and deoxyribose degradation assays. In vitro antioxidant activity of bark fractions in different solvents were assessed by measuring reducing power, DPPH scavenging activity and lipid peroxidation assay and results were compared with standards antioxidants (5)

The green tea flavor compounds described herein may be considered potential antimicrobial agents for cosmetic and food products. For example, antibiotics such as penicillin, erythromycin and tetracycline effectively prevented dental caries in vitro and in vivo but they resulted in derangement of oral and intestinal bacterial floras (6). Aqueous extracts of *A. nilotica* were also shown to have anti-inflammatory, analgesic and antipyretic activity in animal models. *A. nilotica* leaves have been shown to have anti-microbial effects against *C. perfringens* but not against *E. coli* and *S. typhimurium*. It has also been reported that acetone and aqueous extracts of the fruits

and stem bark of *A. nilotica* showed molluscicidal activity against two snail species which transmit schistosomiasis in the Indian population (7). An inflammatory activity involves some macrophages, neutrophils that are known to secrete different mediators that are responsible for the initiation, progression, persistence, regulation, and eventual resolution of the acute state of inflammation (8). The aim of the study is to evaluate the antimicrobial and cytotoxic effect of combined herbal formulation of *Camellia sinensis* (Green tea) and *Acacia nilotica* (Babool).

MATERIALS AND METHODS :

PREPARATION OF THE HERBAL FORMULATION :

To 100 ml of distilled water, 1g of green tea powder and 1 g of babul powder was added. This mixture was heated in heating mantle at 60* c for 15-20mins. Mixture filtered using whatman filter paper. Filtered extract was then condensed to 5 ml.

ANTIMICROBIAL ACTIVITY :

Antibacterial activity of respective nanoparticles against the strain staphylococcus aureus, Bacillus, and E.coli. MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition was measured.

CYTOTOXIC ACTIVITY :

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL,80 µL,100 µL). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for the number of live nauplii . present and calculated by using following formula,

Number of dead nauplii/ Number of dead nauplii+ Number of live nauplii×100

RESULTS AND DISCUSSION :

Green tea polyphenols are also responsible for distinctive aroma, color and taste. Green tea extract can also be used in lipid-bearing foods to delay lipid oxidation and to enhance the shelf-life of various food products (9). Previous studies concluded that polyphenols administered in the form of green tea supplement showed enhanced bioavailability compared with that of green tea, which led to a small but significant increase in antioxidant capacity. Flavanol metabolite formation may have contributed to the antioxidant effect because of the lack of correlation between plasma flavanol concentrations (10).



Fig 1 : Green synthesis of green tea and babul herbal formulation

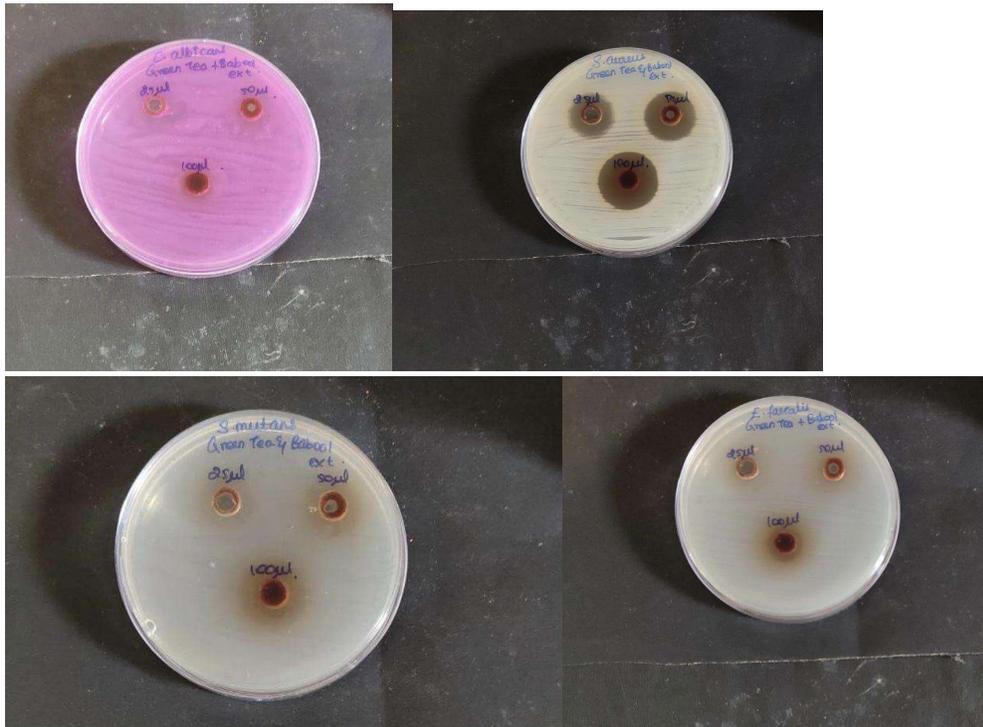


Fig 2 : Antimicrobial activity of green tea and babul herbal formulation

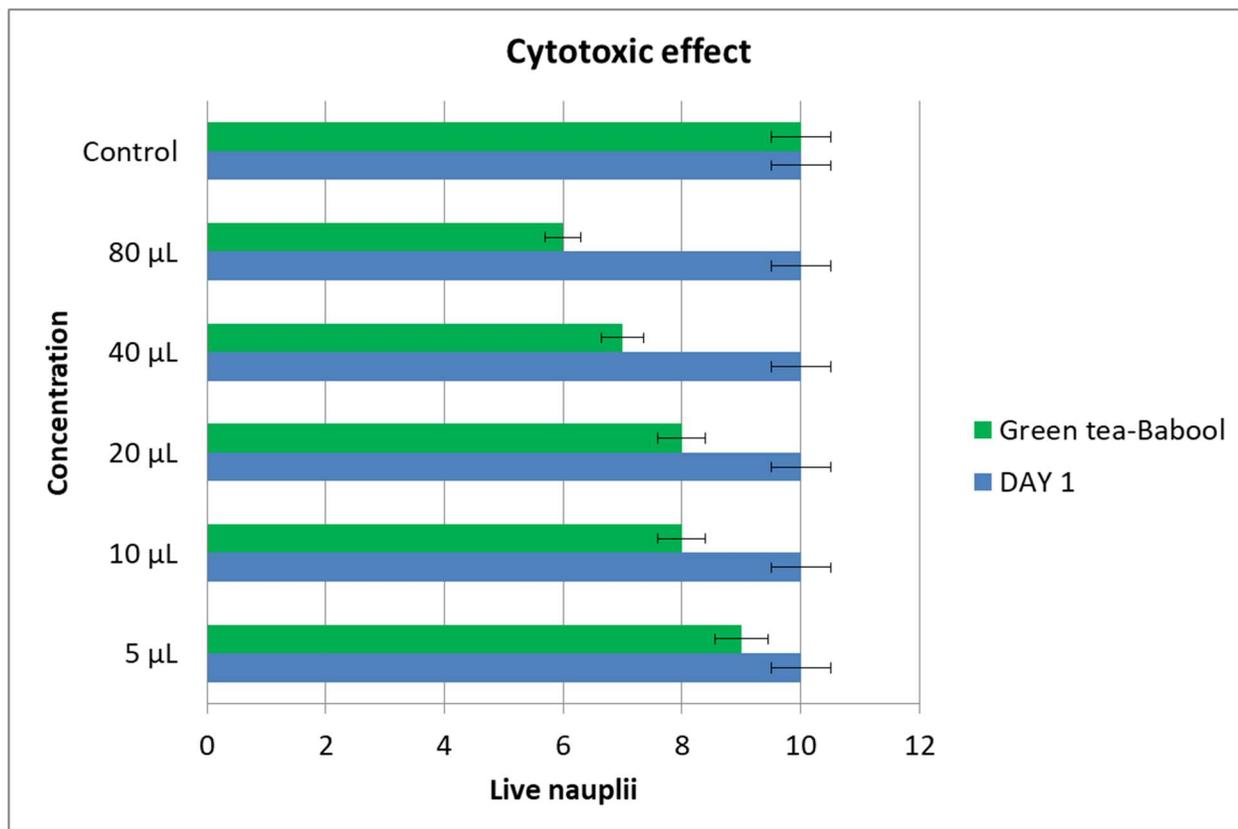


Fig 3 : Cytotoxic activity of green tea and babul herbal formulation

CYTOTOXIC ACTIVITY:

Concentration	Live Nauplii
5 µl	9
10 µl	8
20 µl	8
40 µl	7
80 µl	6
Control	10

Table 1: Cytotoxic activity of the formulation at various concentrations.



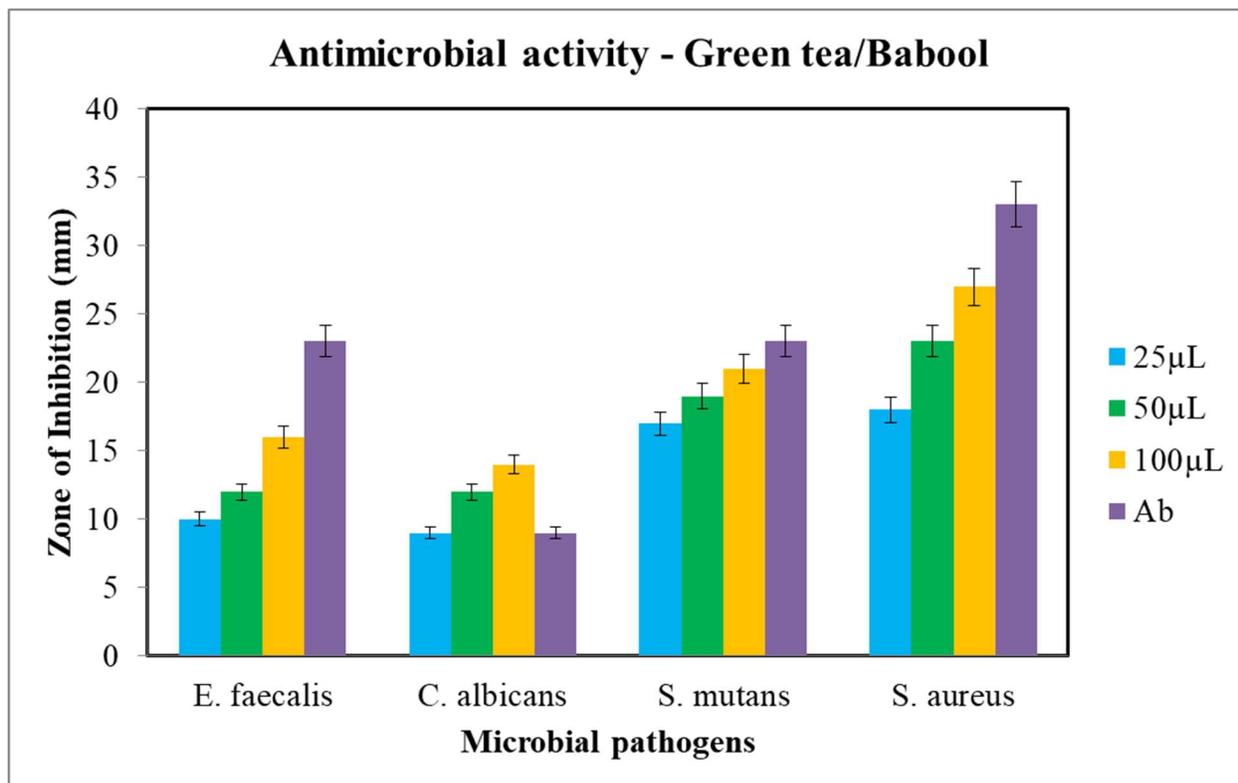
Graph 1 depicts the cytotoxic activity of green tea and babul herbal formulation.

Live naupllis were selected and soaked into the wells that have different concentrations of green tea and babul herbal formulation like 5µl, 10µl, 20µl, 40µl, 80µl and one control group. It has been observed that no change was observed in live nauplius in day 1 and after that differs in each concentration it was effective in 5µl than other concentrations. And there was no change observed in the control group.

ANTIMICROBIAL ACTIVITY

	25µl	50µl	100µl
C. Albicans	9	12	14
S. aureus	18	23	27
S. mutans	17	19	21
E. faecalis	10	12	16

Table 2: Antimicrobial activity of the formulation against microorganisms.



Graph 1- depicts the antimicrobial concentration of green tea and babul herbal formulations. It was found that different concentrations were noted as 20µl, 50µl, 100µl and the control group. Antimicrobial activity was noted in different pathogens like E. faecalis, C.albicans, S.mutans and S. aureus. It was seen that there was an increased zone of inhibition seen in the control group of all pathogens.

Among several plants that can be used in the biogenic synthesis of nanoparticles, green tea (*Camellia sinensis*) was selected due to its rich content of polyphenolic compounds (mainly catechin), which act as reducing and capping agents (11). Previous studies concluded that the green tea flavor compounds described may be considered potential antimicrobial agents for cosmetic and food products. From the study, it has been observed that no change was observed in live nauplius in day 1 and after that it differs in each concentration as it was more effective in 5µl than other such concentrations when tested for its cytotoxic activity. And there was no change observed in the control group in that. Also, When tested for antimicrobial activity, the results were analyzed among different pathogens like E. faecalis, C.albicans, S.mutans and S. aureus which depicts that increased zone of inhibition seen in the control group of all pathogens.

The compounds identified in a common beverage such as green tea should not cause these undesirable side effects (12). Since green tea has been continuously consumed by many people for centuries, either the extract or purified flavor compounds of green tea may be considered safe for practical use in such things as oral care products (13). Babool has many hydrophilic compounds like polyphenols, polysaccharides, and tannis, which explains its antimicrobial activity. It is

relatively high in bioactive secondary compounds. Hence, there is increasing evidence to support this and more promising for drug discovery which has been counted as future scope. Babool also has some other secondary compounds such as diuretic, nutraceutical (polysaccharide and gum), antioxidant (polyphenols), anti-digestive disorder, antiplasmodial, anticancer (14).

Among certain other plant extracts, It has been proved that green tea along with epigallocatechin has an anti-inflammatory effect which improves the quality of life in patients with inflammatory diseases (15). And also has the disadvantage that its overdose can lead to hepatic injury. This article provides the information of combined action of Antimicrobial and cytotoxic activity of herbal formulation of *Camellia sinensis* (green tea) with *Acacia nilotica* (babool). Ours was the first study to evaluate the Antimicrobial and cytotoxic potential of combined herbal formulation of *Camellia sinensis* and *Acacia nilotica*. Since future scope is to provide other such activities other than antimicrobial and cytotoxic effects of green tea and babool herbal formulation.

CONCLUSION :

Since Green tea and Babool have multiple targets, it has the ability to act in a pleiotropic manner, which we may consider their usage to improve the quality of life in patients with inflammatory and microbial disease. Green tea and Babool herbal formulations have beneficial health effects and no severe adverse effects and it has better antimicrobial and cytotoxic effects.

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CONFLICT OF INTEREST :

The authors declare that there is no potential conflict of interest.

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