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The Effect of Nanoparticles Prepared from *Cassia fistula* Linn. Seed Extract on Antibacterial Activity

Research submitted to the Department of Biology, which is a requirement for a Bachelor's degree

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بسم الله الرحمن الرحيم

أَفَرَأَيْتُم مّا تَحْرُثُونَ ۝ أَأَنتُمْ تَزْرَعُونَهُ أَمْ نَحْنُ الزّارِعُونَ ۝ لَوْ نَشَاءُ لَجَعَلْنَاهُ حُطَامًا

صدق الله العلي العظيم

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الإهداء

(وَءَاخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

- إلى المعلم الأول الذي أثار بضياء هداه بصائرنا وأخرجنا من ظلمات الجهل إلى نور العلم وأرشدنا لطريق العلم وبشر السائرين على هذا الطريق بالجنة فقال (ومن سلك طريقاً يلتمس فيه علماً سهل الله له) ..... سيدنا محمد (صلى الله عليه واله وسلم)
- إلى سيدي ومولاي الذي كلما تعثرت وذكرت اسمه نهضت ..... أبا الفضل العباس (عليه السلام).
- إلى من احمل اسمه بكل عز وافتخار لمن مهد لنا الطريق ببذل وتعبه وإلى المرأة التي صنعت مني فتاة طموحة تعشق التحديات والصعوبات قدوتي الأولى إلى من غمرونا بالمحبة وتحملوا معنا عناء الطريق إلى من عاهدتهم بهذا النجاح ها انا أتممت وعدي واهديه لكم .....(امي وأبي)
- إلى الأيادي الطاهرة التي ازالت من طريقي اشواك الفشل، إلى من ساندني بكل حب عند ضعفي، إلى من رسموا لي المستقبل بخطوط من الثقة والحب.....إليكم عائلتي
- لم تكن الرحلة قصيرة ولا الطريق محفوفاً بالتسهيلات لكنني فعلتها فالحمد لله الذي يسّر البدايات وبلغنا النهايات اهدي هذا النجاح لنفسي الطموحة اولاً، إلى نفسي العظيمة القوية التي تحملت كل العثرات وأكمل رغم الصعوبات، ابتدأت بطموح وانتهت بنجاح، ثم إلى كُل مَن سعى معي لإتمام مسيرتي الجامعية

والله ولي التوفيق

جمانه، حوراء، تبارك

شكر وتقدير

الحمد لله حمد كثيراً حتى يبلغ الحمد منتهاه والصلاة والسلام على أشرف مخلوق أناره الله بنوره واصطفاه

وانطلاقا من باب من لم يشكر الناس لم يشكر الله أتقدم بخالص الشكر والتقدير للأستاذ المشرف أ.د. هدى جاسم التميمي على إرشاداتها وتوجيهاتها التي لم تبخل بها علينا يوما، كما أتقدم بجزيل الشكر والعطاء إلى كل يد رافقتنا في هذا العمل سواء من قريب أو من بعيد والشكر موصول كذلك إلى أوليائنا الذين سهروا على تقديم لنا كل الظروف الملائمة لإنجاز هذا العمل.

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جمانه، حوراء، تبارك

Abstract

This study evaluates the antimicrobial effects of AgNP suspensions from aqueous extracts of *Cassia fistula* L. seed (Fabaceae family) against several microbes representing gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using standard agar disc diffusion technique. Inhibition zones showed that in the concentration of 125 µg/ml, no inhibition was shown for all types of bacteria in hot water extract and in both bacteria *E.coli* and *Staphylococcus aureus* at the same concentration in cold water extract and the results showed an apparent increase in the rate of the diameter of the inhibition zones by increasing the concentration of each of the plant extracts towards the growth of bacteria .

The current research suggests that the plant includes flavonoids, terpenoids, and steroids that may be useful in creating phytomedicine to treat the examined bacterial illnesses. This investigation showed that an aqueous extract of *C.fistula* seed had significant antibacterial activity against serious clinical infections.

1: Introduction

Over the last few years, researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. Interestingly it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. It is worth mentioning that medicinal plants are considered a vast source of several pharmacological principles and compounds that are commonly used as home remedies against multiple ailments (Maity *et al.*, 2009)

Medicinal plants currently occupy a large place in industrial production as they are a major source of good, plant-based drugs, as they are the primary source of active ingredients used to produce some of the primary chemical compounds for the drug industry, which give them their medicinal action (Tipu *et al.*, 2002).

The evaluation of these drugs is primarily based on phytochemical, and pharmacological approaches, including various instrumental techniques such as chromatography, microscopy and others. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different healthcare systems, the evaluation of the rich heritage of traditional medicine is essential (Gupta, 2010).

Natural plants are nature's gift having medicinal importance. They help humans to lead healthy lives. Those plants that are wider in distribution help us a lot with the remedy of different diseases. Here some traditional systems are Siddha, Ayurveda and Homeopathy Unani. Traditionally many plants are being evaluated and used. One of them is *Cassia fistula* L.

Cassia fistula is a flowering plant that belongs to the subfamily Caesalpiniaceae of the leguminous family (Fabaceae) commonly known as Amaltas. Out of the 400 species, it comprises the genus, *Cassia*. It is widely distributed in tropical countries of the world India, China, Ceylon, Egypt,

Mauritius, South Africa, Mexico, Brazil, East Africa, Thailand, and Sri Lanka. *Cassia fistula* is used traditionally for the treatment of many diseases but now scientific research enables us to explore the hidden secondary metabolites and their role towards the organisms (pharmacological activities) (Gupta *et al*., 2000; Jehangir *et al*., 2010).

Cassia fistula is employed in the management of different conditions including and not limited to these: relieving the symptoms of asthma, leprosy, ringworm, heart related disorders and fever. Extracts from *C. fistula* is used as laxative as well as in constipation management; root is employed in treating of flus and colds whereas the leaves are employed in relieving pain, edema, and reducing skin irritation as result of swelling. Additionally, extracts of the stem bark and fruits are used in eliminating toxins from the blood (Jung *et al*., 2016).

Cassia fistula exhibits many applications in therapy particularly in the traditional medicine system. This is an alien species, and it is popularly used as an ornamental plant because of its attractive yellow flowers. Seeds from this species are used as medicine for treating gastritis and diarrhea; they are likewise used as insect repellent. The seeds are also used to treat biliousness in addition to improving appetite. The roots are used in the treatment of skin disorders, syphilis, leprosy, and tuberculosis (Pawar and Killedar, 2017).

Scientific classification of plants (according to Maqsood *et al*., 2020)

Kingdom: Plantae
Subkingdom: Tracheobinota
Super Division: Spermatophyta
Division: Mangoliophyta
Class: Magnoliopsida
Sub Class: Rosidae
Order: Fabales
Family: Fabacae
Genus: *Cassia*
Species: *fistula* L.

Synonyms: *Cassia exceisa* kunth. and have many vernacular names such as in Arab: Khayarsambhar chaiyaphruek, khuun*;* English: golden shower, Indian laburnum*;* Hindi: Bandarlathi, bharva, suvarnaka and as Trade name: Indian laburnum (Bhalerao and Kelkar, 2012).

Botanical Description

It is a deciduous tree with greenish grey bark, compound leaves, the leaves are 1 to 1.5 m long, opposite, pinnate. Leaflets 7 to 15 cm long and 2.5 to 3 cm in width, ovate or ovate-oblong, acute, stipules minute, linear-oblong, obtuse, pubescent. bright green and glabrous above, paler and silvery pubescent beneath when young, the midrib densely pubescent on the underside, base cuneate; main nerves numerous, close, conspicuous beneath; petioles 6-10 mm long, pubescent or glabrous (Kirtikar and Basu, 2006). Flowers are bright yellow and appear in graceful hanging clusters. Flowers in lax racemes 30-50 cm. long; pedicels 3.8- 5.7 cm. long, slender, pubescent and glabrous. Calyx 1 cm long divided to the base, pubescent; segments oblong, obtuse. Corolla 3.8 cm across, yellow; stamens all antheriferous (Khan and Alam, 1996).

Cassia fistula has cylindrical fruit which is pendulous septate and brown. The length of the fruit is about 25 to 45 cm in diameter is about 1-3 centimeter. Seeds are reddish brown in color and lenticular in texture. The stem bark is brown in color (Bhatnagar *et al*., 2010).

Habitat and Geographical distribution:

The plant usually prefers deep, well-drained, moderately fertile sandy loamy soil, but can also grow on calcareous, red, and volcanic soils. The plant is usually found growing in the rain forest, seasonally dry forest, woodland, riverine, gallery forest, wooded grassland, on dry scrub, thickets and coastal areas, low altitudes, gardens, parks, and even on urban lands.

There are more than 500 species of Amaltas around the world (Pawar *et al.*, 2017)

In deciduous and mixed monsoon forests throughout greater parts of India, ascending to 1300 m in the outer Himalayas. In Maharashtra, it occurs as a scattered tree throughout the Deccan and Konkan (Gupta *et al.*, 2000). The plant is cultivated as an ornamental throughout India (Pawar *et al.*, 2017)

2: Materials and Methods

A- Tools and instruments

Laboratory Tools Used in the Current Study with the Name of the Manufacturer and Origin Country are listed in Table 1.

Table 1: Laboratory Devices and Tools are used in current study.

No.	Tools name	Company and origin
1	Beaker	Lab (Germany)
2	Centrifuge tubes	Afco-Dispo (Jordan)
3	Cotton	Aslanli (Turkey)
4	Eppendorf tube	Pioneer (South Korea)
5	Micropipettes (different sizes)	CYAN (Belgium)
6	Gloves	Broche (Turkey)
7	Class Cylinder Graduated	Lab (Germany)
8	Filter paper	Whatman No.1
9	High-speed cooling Centrifuge	Hettich/ Germany
10	Shaker Incubator	Genex / USA
11	Oven	Hisense/ China
12	Disposable plastic Petri dishes	Afco/ Jordan
13	Sterile swab	Lab. service / S.P. A
14	Sensitive Electronic Balance	Kern / Germany
15	Refrigerator	Hitachi / Japan

B- Chemicals

Laboratory Tools Used in the Current Study with the Name of the Manufacturer and Origin Country are listed in Table 2.

Table 2: Laboratory chemicals, instruments and disposable materials

No.	Material name	Volume / Concentration
1	AgNo3	1mM
2	Nutrient agar	Accumax
3	nutritional broth	Accumax
4	deionized water	

C- Methods:

I: Preparation of the Extracts of Cold and Hot Water from a Plant

According to Harborne's maceration method (Figure 1), the cold and hot water extract was prepared from *Cassia fistula* seed (1998)

1. Macerate 100 grams of plant material in a beaker with 1000 ml of cold and boiling water separately, stirring continuously for 30 minutes.
2. Cover tightly to prevent any foreign materials from entering and leave for 24 hours to decompose the active ingredients more effectively.
3. Filter this solution through two layers of gauze and centrifuge them for 10 minutes at 3000 rpm.
4. Take the leachate and leave the residue, then place the solution in (40-45) C° an electric oven to acquire dry material from the extract.
5. Keep refrigerated in glass containers until ready to use. The process was repeated numerous times to obtain a significant amount of the extract to conduct experiments.
6. Prepare the stock solution concentration equivalent to 300 mg/ml after 7.5 g of the crude plant was dissolved in 25 ml of distilled water.

Figure 1: Fruit and seed of *Cassia fistula*

II: Preparation of silver nanoparticles from *C.fistula* seed extract.

Silver nanoparticles were made by combining 5mL of aqueous extract of *Cassia* seed with silver nitrate solution (weight 0.017g silver nitrate, dissolved in 100 mL deionized water) and put in an incubator shaker for 24 hours. The brown tint suggested the production of silver nanoparticles, which then began to turn dark brown. The change in color shows the creation of AgNPs. The solution was held at room temperature for 24 hours to ensure the nanoparticles were utterly stable. The particles were separated after this time by centrifugation at 15000 rpm for 15 minutes, followed by washing with deionized water, freezing the powder, and using it for additional analyses (Choudhary *et al.*, 2015).

Fill a dish with the solution and let it dry in the dark before collecting the powder, the experiment was carried out under settings, including silver ion concentration to find the best conditions for nanoparticle production.

III- Antibacterial effect study

The antibacterial activity of *C.fistula* seed AgNP suspensions on gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were investigated in this study, employed the following procedures to conduct these tests, according to (Prastiyanto *et al.*, 2020) by the Well diffusion method to the determination of the inhibition zone.

1. Dilutions of AgNPs at 125, 250, 500, and 1000 µg /ml were prepared from a stock concentration
2. For this experiment, indicators bacteria were activated for 18 hours at 37°C in nutritional broth, and the turbidity tube was 0.5. The MacFarland technique was used to determine turbidity.
3. A cotton swab was used to cultivate bacteria on sterilized nutrient agar plates.
4. Wells were cut out with the end of a sterilized pasture pipette after 5-10 minutes.
5. Solution of AgNPs 100µl of each concentration was applied to wells and incubated for 24 hours at 37° C.
6. Inhibition zones were measured in mm.

3: Results and Discussion

Two types of extracts were prepared from *C.fistula seed* which are hot water extract, cold water extract, and prepare silver nanoparticles from each *C.fistula* seed extract different concentrations of these extracts (125, 250, 500, and 1000 µg /ml) were used to detect their inhibitory effect on different types of bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*).

From Table 3 The results of the study of the inhibitory effectiveness varied according to the type of extract and the different types of bacteria, and there was an apparent increase in the rate of the diameter of the inhibition zones by increasing the concentration of each of the plant extracts towards the growth of bacteria, as the diameters of the zones of inhibition of the hot aqueous extract towards the growth of bacteria ranged between 20mm in *Pseudomonas aeruginosa* at a concentration of 1000 µg/ml (Figure 2-A) to 11mm appeared in both *Streptococcus pyogenes* and *E.coli* at a concentration of 250 µg/ml (Figure 2-B and C), While in the concentration of 125 µg/ml explained no inhibition was shown for all types of bacteria, and likewise, there was no inhibition at the concentration of 250 µg/ml for *Staphylococcus aureus*.

Table 3: Diameters of inhibition zone for each type of *C.fistula* seed AgNPs against four species of bacteria

No	Material	Concentration	Gram positive bacteria		Gram negative bacteria	
			Streptococcus pyogenes	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***E.coli***
1	Control	Control	zero	Zero	zero	Zero
2	Hot water	1000 µg/ml	19	17	20	18
3		500 µg/ml	14	12	16	14
4		250 µg/ml	11	Zero	12	11
5		125 µg/ml	zero	Zero	zero	Zero
6	Cold Water	1000 µg/ml	21	16	19	20
7		500 µg/ml	18	13	14	14
8		250 µg/ml	13	Zero	11	11
9		125 µg/ml	11	Zero	10	Zero

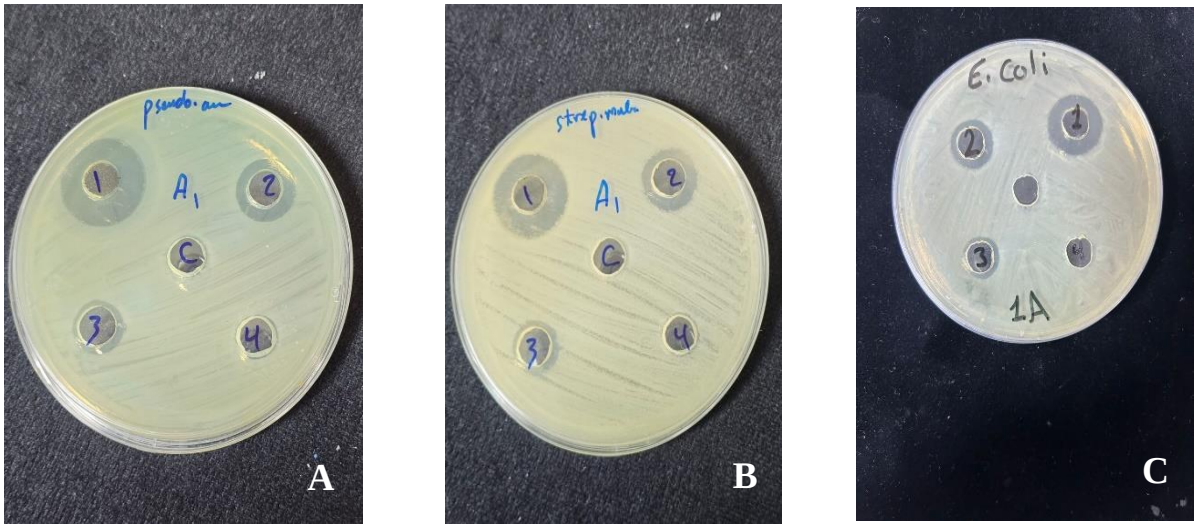

Figure 2: The antibacterial activity of *C.fistula* seed AgNP suspensions from hot extract (A- *Pseudomonas aeruginosa*, *B- Streptococcus pyogenes*, *C- E.coli)*

In cold water extract of *C.fistula* seed AgNP suspensions results were shown the greatest inhibition zone approximately at 21mm was seen against gram-positive bacteria specifically *Streptococcus pyogenes* at a concentration of1000 µg/ml (Figure 3-A) but the least inhibition zone approximatlely 10 mm was appearded in *Pseudomonas aeruginosa* at a concentration of 125 µg/ml (Figure 3-B). In addition, the results were showed no inhibition zone in both bacteria *E.coli* and *Staphylococcus aureus* at a concentration of 125 µg/ml (Figure 3- C and D), bacteria *Staphylococcus aureus* at a concentration of 250 µg/ml (Figure 3-D).

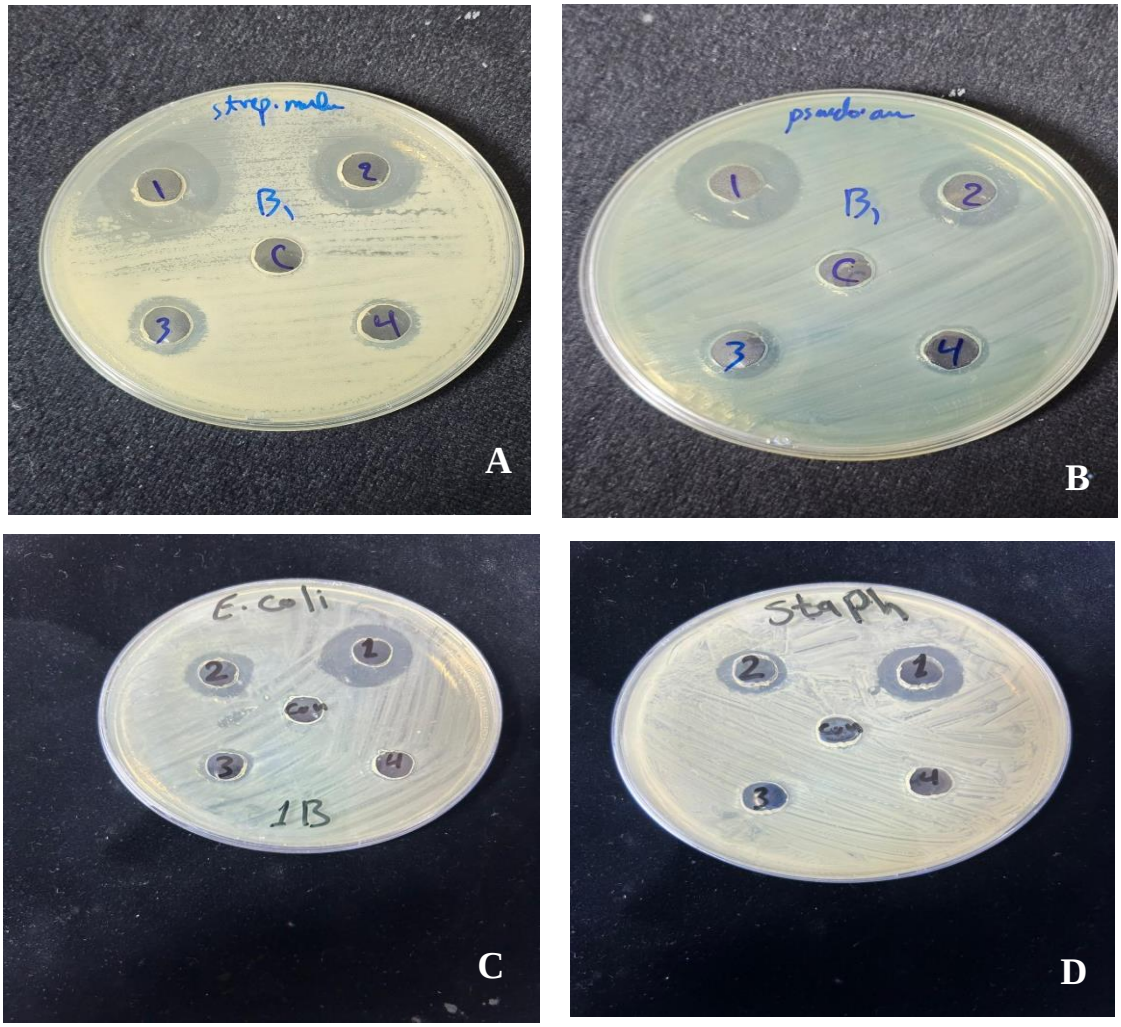

Figure 3: The antibacterial activity of *C.fistula* seed AgNP suspensions from cold extract (A- *Streptococcus pyogenes*, *B-Pseudomonas aeruginosa* , *C- E.coli*, *D- Staphylococcus aureus)*

the *Cassia* plant showed its ability to biosynthesis silver nanoparticles after adding silver nitrate (AgNO3) at a concentration of 1mM to the *Cassia*, The color change in the reaction mixture after 24 hours of incubation at 37°C in a vibration incubator that reflected clear evidence of the formation of silver nanoparticles as a result of ionic reduction (Ag+) to elemental silver (Ag0) through a group of reducing agents in *Cassia* seed is eco- friendly and this result was confirmed by (Mohammed *et al*., 2014). The transition from light brown to dark brown pointed to the synthesis of silver nanoparticles (Korbekandi *et al*., 2013; Benakashani *et al*., 2016).

According to many theories Ag-NPs may adhere to the surface of cell membrane, altering the cells permeability and respiration functions, and also the significant antibacterial activity of nanoparticles is due to their large surface area, which provides more surface area for contact with organisms than big particles. Furthermore, Ag-NPs may interact not only with the membrane's surface but also with the bacteria inside (Sahayaraj, 2011).

Kuo and *et al*., (2002) confirmed three lectins from the *Cassia fistula* seeds possess antibacterial activities against various pathogenic bacteria, and antibacterial activity of the aqueous and alcoholic extract of the stem bark was highly effective. Also, Verma (2016) mentioned the *Cassia fistula* have a rich source of tannins, flavonoids and glycosides therefore it’s had pharmacological activities include antidiabetic, antibacterial, antifertility, anti-inflammatory antioxidant, hypatoprotective, antitumor, antifungal activities.

Danish and *et al*. (2011) mentioned that is widely used in traditional medicinal systems of India and has been reported to possess hepatoprotective, anti-inflammatory, antitussive, antifungal and also used to check wound healing and antibacterial. It is known as a rich source of tannins, flavonoids and glycosides. The innumerable medicinal properties and therapeutic uses of *Cassia fistula* as well as its phytochemical investigations prove its importance as a valuable medicinal plant.

In previous studies, the antibacterial and antifungal activities of solvent extracts of *C. fistula* were tested against *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and fungal strains *Aspergillus clavatus, Aspergillus niger*, *Candida albicans*, showing moderate to strong activity against most of the organisms tested (Bhalodia *et al*., 2012).

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