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ANTIMICROBIAL AND ANTIVIRAL PROPERTIES OF PLANT EXTRACTS AND ISOLATED COMPOUNDS IN VITRO

Sameera Begum[1], Dr. Shilpi Shrivastava[2]
[1]Research Scholar, Department of Chemistry, Kalinga University, India.
[2]Professor, Department of Chemistry, Kalinga University, India.

Abstract:
The different extracts have been assessed for their potential in vitro antibacterial and antiviral capabilities. The essential oil was analyzed by GC-EIMS, resulting in the discovery of 92 components that accounted for 96.1% of the oil. The primary constituents included 1,8-cineole, a-pinene, caryophyllene oxide, and sabinene. The hexane and dichloromethane extracts exhibited no antibacterial action. The methanolic extract and essential oil shown varying levels of effectiveness against the bacteria tested. The antiviral efficacy of the plant samples was evaluated using two model systems: the replication of two influenza viruses in MDCK cells and the replication of two herpes simplex viruses in MDBK cells. The methanol extract had a potent antiviral action against the influenza virus, resulting in a considerable reduction in the growth of both A/Weybridge and A/Aichi strains. This extract also demonstrated antiviral efficacy against the herpes virus. The concentration of total phenolics in the methanol extract was significantly high. Dichloromethane was the subsequent substance. This extract has also been discovered to contain a high concentration of flavonoids. An affirmative association was detected between the potential for biological activity and the quantity of phenolic chemicals present in the extracts.
Key words: antimicrobial, antiviral, extract.

INTRODUCTION

The genus Salvia is a highly prevalent member of the Lamiaceae family, with approximately 900 species found worldwide. It is known for producing a wide range of valuable secondary metabolites, such as terpenes and phenolics and their derivatives. These compounds have been extensively utilized in the pharmacopoeias of numerous countries (Tepe et al., 2007). The name of this genus, "salvus," in Latin means "safe" or "well." The reputation of Salvia species for its healing properties may be traced back to ancient Roman times. One particularly well-known species, Salvia officinalis (often known as sage), is responsible for this reputation (Könemann, 1999). Several research groups have conducted numerous studies on various biological activities of certain Salvia species, including S. officinalis L. and S. miltiorrhiza Bunge (Chinese Dansheng). These studies have provided evidence of their potential efficacy in treating conditions such as coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrhea, and neuroasthenic insomnia (Li, 1998; Lu and Foo, 2002). Herbal treatments have been used throughout human history to cure several infectious disorders. Plant materials still have a significant role in basic health care as medicinal medicines in many impoverished nations, even in modern times. Plants remain the primary source of pharmaceuticals for most of the world's population. Approximately 25% of prescription medications in western medicine are derived from higher plants. Out of the 121 bioactive plant-derived chemicals now in global usage,

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74% were discovered via research inspired by ethnomedicine (Anyinam, 1995). Recent studies have shown that higher plants has the potential to serve as a source of novel antibacterial compounds. Scientists have shown tremendous interest in screening plant extracts to develop novel medications that are efficient in treating various ailments. There have been several reports in the literature on the testing of plant extracts from medicinal plants for their antibacterial properties. However, most of these findings have not been thoroughly assessed (Sokmen et al., 1999). This statement holds true, especially when considering the Turkish flora, which boasts one of the largest collections of plant species in continental Europe, with about 9000 species of flowering plants (Davis, 1965 - 1984). Due to its advantageous geographical location, the combination of traditional medicine knowledge from both Western and Eastern cultures has resulted in this area having a diverse heritage in using medicinal plants (Gozler, 1993). The phenolic compounds extracted from this species have been shown to be very effective as antibacterial agents. The detailed discussion focused on the existence of phenolic acids, particularly caffeic acid and its metabolites, as well as flavonoids in several Salvia species. Particularly, rosmarinic acid, also known as a-Ocaffeoyl-3,4-dihydroxyphenyllactic acid, is very intriguing due to its significant biological properties (Tepe, 2007). Elsewhere, the antioxidant properties of several species belonging to the genus Salvia have been observed. Previous research on the biological activities of Salvia species endemic to the Turkish flora have shown that this genus has significant potential, particularly in antioxidant systems, for the food and cosmetic sectors (Tepe et al., 2004, 2005a, 2005b, 2006, 2007). In addition to phenolic compounds, the analysis of terpenes in the essential oils of Salvia species and their potential pharmacological effects are also highly regarded (Lu and Foo, 2002). While there are a large number of Salvia species in the Turkish flora, with 88 species and 93 taxa, 45 of which are indigenous (Guner, 2000), only a few of them have been studied for their biological activities and bioactive characteristics. The majority of these species remain unexplored in the literature. Based on our comprehensive literature investigation, we have found no existing studies on Salvia cedronella. Yesilyurt et al. (2008) have only documented the antioxidant capacity and phenolic components of this species in one instance. The objective of this work was to assess the in vitro antibacterial and antiviral properties of the essential oil and several extracts of S. cedronella, a plant native to Turkey. Additionally, the study aimed to identify the chemical makeup of its essential oil, which has not been previously determined.

BACKGROUND

Traditional medicine is widely practiced in Africa, with about 75% of the population seeking the expertise of traditional medical practitioners (TMPs), mostly traditional physicians, for medical issues. The primary reason for this is because the traditional healthcare system is readily available, culturally accepted, and more affordable compared to expensive conventional treatment. Medicinal herbs are often used in Nigeria and other underdeveloped nations for the treatment of many illnesses, particularly infectious infections [1].

Medicinal plants are commonly recognized as a reliable and efficient source of safe medicines that have the potential to produce new drugs. However, this potential is currently at risk due to the concerning loss of biodiversity. Recent estimates suggest that one out of every five plant species on Earth is in danger of becoming extinct [2]. Therefore, experts in the field of drug discovery are now making a

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pressing endeavor to systematically study and investigate the bioactivity of medicinal plants that are traditionally employed in different ethnobotanical practices. The aim is to create a connection between the traditional medicinal use of these plants and their potential in the development of contemporary drugs [3, 4].

Enteroviruses (EVs) are a group of RNA viruses belonging to the family Picornaviridae. They have a single positive-stranded genomic RNA and are comprised of over 100 different serotypes. They have been recognized as significant causative factors for encephalitis, particularly in children and adults. Enteroviruses, including coxsackievirus A9, A10, and B5; echoviruses 4, 5, 9, 11, 19, and 30; and EV 71, 75, 76, and 89, have been documented in cases of encephalitis and outbreaks worldwide [5]. Echoviruses, also known as enteric cytopathic human orphan viruses, are tiny non-enveloped icosahedral viruses that have a diameter of around 300 Å (27 nm). They are classified as part of the enterovirus species B within the Picornaviridae family and consist of 29 different serotypes (E1-E29). Echoviruses often cause short-term fever, perhaps accompanied by a rash or moderate symptoms in the upper respiratory system [6]. Echovirus infections may lead to several clinical symptoms, such as aseptic meningitis, paralysis, encephalitis, ataxia, Guillain-Barré syndrome, exanthema, respiratory illness, diarrhea, pericarditis, myocarditis, and hepatic dysfunction.

Echoviral infection, similar to other enteroviruses, is transmitted by the fecal-oral pathway [7]. Currently, there is no particular pharmacological intervention being used in clinical practice for enteroviruses. Additionally, the creation of a vaccine that targets all enterovirus serotypes is not currently possible owing to the large number of serotypes. In addition, there is the overarching issue of viral resistance to antiviral medications, particularly RNA viruses, which have the ability to change frequently and quickly develop strains that are resistant to drugs [10]. Hence, it is crucial to seek for novel and potent drugs that possess anti-enteroviral action. Medicinal herbs have shown their efficacy in combating a diverse range of viral infections by impeding the reproduction process of several DNA and RNA viruses [11]. This study confirmed the effectiveness of using plant-based antivirals in several traditional medical practices [12]. Thus, the objective of this study was to assess the antiviral properties of 27 plant extracts against echovirus serotypes 7, 13, and 19.

RESEARCH METHODOLOGY

Botanical specimens and the process of extracting their components, A total of 27 distinct morphological components derived from 26 plants (as shown in Table 1) were gathered from different areas in Ibadan, located in the South-west region of Nigeria. These components were specifically chosen due to their ethnobotanical significance in treating infectious illnesses. The plants were then identified and verified at the Forestry Herbarium Ibadan (FHI). The plant components were dried in the air, crushed into a powder, and then soaked in methanol for 72 hours at room temperature (26–33 °C). The extracts obtained were filtered and then concentrated using a rotary evaporator under vacuum.

Separation of the potent extract into its constituent parts, The crude extract with the highest level of activity, obtained from Macaranga barteri, was separated into different fractions utilizing liquid-liquid partitioning and vacuum liquid chromatography (VLC) using a process known as bioassay guided fractionation. In summary, 150 g of the raw methanol extract of M. barteri was dissolved in 250 mL of

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a solution consisting of 70% methanol and water. The resulting mixture was then separated into several solvents, namely n-hexane, dichloromethane (DCM), ethylacetate (EtOAc), and aqueous methanol, each in quantities of 400 mL repeated four times. The DCM fraction, which exhibited the greatest antiviral activity, underwent vacuum liquid chromatography (VLC) on silica gel employing a gradient elution solvent system consisting of n-hexane/EtOAc (10:1 to 5:5, each 250 mL). The fractions obtained were grouped into six sub-fractions (DCMA - DCMF) based on the similarity of their analytical TLC characteristics. The VLC fractions were treated with chromogenic spray reagents, such as 1% vanillin/sulphuric acid and aluminium chloride (AlCl3), to identify the potential chemicals that belong to different classes.

Viruses and cell lines

The research collected three serotypes of echovirus (E7, E13, and E19) from stool isolates at the WHO Polio Laboratory, Department of Virology, University of Ibadan, Nigeria. The viruses were kept at a temperature of -70 °C until they were used. The human rhabdomyosarcoma (RD) cells were acquired from the Centre for Disease Control in Atlanta, Georgia.

The cells were cultivated in Eagle's minimum essential medium (MEM; Sigma-Aldrich) with the addition of 10% fetal bovine serum (FBS), 100 units/mL of penicillin, 100 µg/mL of streptomycin, 2 mM L-glutamine, 0.07% NaHCO3, 1% non-essential amino acids, and a vitamin solution. The cells were incubated in a humidified incubator at a temperature of 37 °C and a CO2 concentration of 5% for a duration of 72 hours. The cytotoxic tests and antiviral assays were conducted using a test medium that consisted of just 2% fetal bovine serum, also known as maintenance medium.

Curcumin, also known as diferuloylmethane, is a polyphenol with a low molecular weight (Priyadarsini, 2014). It has been used for millennia in traditional Asian medicine to cure many disorders. Curcumin is extracted from the rhizome of Curcuma longa L., which is often known as turmeric. Multiple investigations have shown that curcumin has a broad spectrum of biological and pharmacological characteristics. Evidence has shown its efficacy against several significant human infections, such as those belonging to the Staphylococcus, Streptococcus, and Enterococcus genera. Several studies have investigated the effectiveness of Curcuma longa L. extract against staphylococci. The data from these studies have shown that curcumin has antimicrobial activity against both methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible Staphylococcus aureus (MSSA). The minimum inhibitory concentrations (MICs) of curcumin were found to be in the micromolar range. These findings have been reported by Mun et al. (2013), Teow and Ali (2015), Hung et al. (2020), Jaiswal and Mishra (2018), and Sardi et al. (2017). In addition, a combination of Curcuma longa L. extract and different antibiotics (oxacillin, ampicillin, ciprofloxacin, gentamicin, amikacin, polymyxin B, and norfloxacin) showed a synergistic effect in a strain-specific manner. No antagonistic effects were observed in the studies conducted by Mun et al. (2013), Teow and Ali (2015), and Betts et al. (2016). The potential synergistic effects may arise from curcumin's capacity to bind bacterial enzymes, leading to a decrease in the breakdown and degradation of antibiotics (Zhou et al., 2011; Teow and Ali, 2015). The curcumin that was free and microencapsulated exhibited bacteriostatic effects against Bacillus subtilis and Bacillus cereus (Jaiswal and Mishra, 2018; Praditya et al., 2019). Curcumin shown efficacy against Streptococcus pyogenes. Furthermore, when coupled with polymyxin B, a synergistic effect was

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seen (Betts et al., 2016).
The sensitivity of Listeria innocua to UVA-light exposed curcumin was assessed, and a synergistic impact was seen, even at low doses of curcumin (de Oliveira et al., 2018). Curcumin has been shown to block Sortase A from Staphylococcus aureus. Sortase A is an important enzyme in Staphylococcus mutans, responsible for the covalent attachment of the main cell surface adhesin to the cell wall. This enzyme plays a crucial role in the production of biofilms (Hu et al., 2013).
Essential oils are intricate combinations of several components and have been well recognized for their ability to inhibit the growth of microorganisms.
Drug combinations are commonly used in antimicrobial treatment because they enhance effectiveness by working together in a synergistic or additive manner. This allows for lower doses of medication, reducing costs and minimizing adverse side effects. Additionally, drug combinations can broaden the range of activity against different pathogens (Morlock et al., 2014; Bag and Chattopadhyay, 2015).

Plant material and preparation of the extracts

In July 2007, S. cedronella was obtained from Burdur, Turkey. The preparation of extracts from air-dried and ground plant materials included the use of solvents with different levels of polarity. The extraction methodology for each solvent is shown below. 100 grams of dried plant material from S. cedronella were extracted using hexane (yield: 2.38% w/w), followed by dichloromethane (yield: 3.08% w/w) and methanol (yield: 13.80% w/w) in a Soxhlet device. Each solvent was used for 6 hours. (Tepe et al., 2005a).
The obtained extracts were freeze-dried and stored in a light-protected environment at a temperature of 4 degrees Celsius until they were used.

Extraction of the essential oil

The plants' aerial parts, which had been air-dried and crushed, were subjected to water distillation for a duration of 3 hours using a clevenger-type equipment. The resulting yield was 0.08% (v/w). The obtained essential oil was dehydrated using anhydrous sodium sulfate and then kept at a temperature of +4°C until it was tested and evaluated.

Antimicrobial activity

The extracts and essential oil obtained from S. cedronella were individually tested against various bacteria and fungi, including Bacillus cereus, B. subtilis, Enterobacter aerogenes, E. faecalis, Ecsherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, P. fluorescens, Staphylcoccus aureus, S. epidermidis, Listeria monocytogenes, Proteus mirabilis, and Candida albicans. To test the effectiveness of the extracts and oil, disc-diffusion, microwell dilution, and MIC agar dilution methods were used, following the methodology described in a previous study (Gulluce et al., 2003). Positive reference standard antibiotic discs containing ofloxacin (10 µg/disc), sulbactam (30 µg) + cefoperazona (75 µg) (105 µg/disc), and netilmicin (30 µg/disc) were used. Additionally, amphotericin B was used as a reference antibiotic in the microwell dilution method (Sigma).

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Antiviral activity

The samples were subjected to treatment with DMSO, DCM, or hexane, as appropriate, and then diluted in bi-distilled sterile water to obtain 10% solutions. For the studies, the cell culture medium was diluted in a 2-fold manner just before use. Rimantadine hydrochloride (Rimantadine) was acquired from Hoffman - La Roche Inc., located in Nutley, NJ, USA. (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) was received from Sigma-Aldrich Chemie GmbH in Deisenhofen, Germany.

Madin-Darby canine kidney (MDCK) and Madin-Darby bovine kidney (MDBK) cells were grown in Dulbecco's eagle medium (GibcoBRL, Scotland, UK), supplemented with 5% fetal calf serum (FCS) (BioWhittaker Europe, Germany) and antibiotics. The cell cultures were incubated at 37°C with 5% CO2 until they formed a complete layer of cells. 0.5% FCS was included in the antiviral tests.

Viruses

The chicken influenza virus A/ch/Germany/34, strain Weibridge (H7N7) and the human influenza virus A/Aichi/2/68 (H3N2) (A/Aichi) were cultivated in MDCK cells with 2 µg/ml trypsin (Sigma). The infectious titer was between 105.7 - 107 TCID50/ml (50% tissue culture infectious doses/ml), and the hemagglutination titer was 1024. Herpes simplex virus type 1 (HSV-1), strain DA, and herpes simplex virus type 2 (HSV-2), strain Bja, were grown in MDBK cells with an infectious titer of 1016 - 1018 TCID50/ml. These strains were obtained from the collection of the Institute of Microbiology, Bulgarian Academy of Sciences, Sofia. The virus stocks were stored at a temperature of 700C.

RESULTS AND DISCUSSION

Amount of total phenolics and flavonoids

The total phenolic test was conducted by measuring the absorbance values of different extract solutions after reacting them with the Folin-Ciocalteu reagent. The absorbance values were then compared to the absorbance values of standard solutions containing pyrocatechol equivalents, as previously explained. The data collected from the total phenolic test confirms the significant involvement of phenolic compounds in scavenging free radicals and/or reducing systems. The methanol extract exhibited a very high level of total phenolics, measuring 106.64 ± 1.33 µg GAEs / mg extract, as anticipated. The dichloromethane extract had a value of 42.98 ± 2.72 µg GAEs / mg, as shown in Table 2. It is crucial to note that there was a strong positive link between the biological activity potential and the quantity of phenolic chemicals in the extracts. Conversely, the dichloromethane extract has been discovered to have a high concentration of flavonoids, namely 41.26 ± 0.84 µg QEs / mg. Yesilyurt et al. (2008) previously described the antioxidative activity potential, total phenolics, and flavonoids of this plant. Based on the findings, the acetone extract of S. cedronella exhibited a significant amount of total phenolics, whereas the flavonoid concentration was determined to be low. The results obtained from this investigation strongly align with the findings of the current study.

Antimicrobial activity

The antimicrobial properties of the essential oils and extracts with different polarities were tested against a diverse range of human, plant-associated, and foodborne microorganisms. The effectiveness of these

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substances was evaluated both qualitatively and quantitatively by examining the presence of inhibition zones, measuring the diameter of these zones, and determining the minimum inhibitory concentration (MIC) values (Table 1). The hexane and dichloromethane extracts exhibited no antibacterial action. Regarding the methanol extract, B. cereus, B. subtilis, E. aerogenes, E. faecalis, S. aureus, S. epidermidis, P. mirabilis, and C. albicans exhibited different levels of sensitivity. The growth inhibitions of these microorganisms varied between 31.25 mg/ml and 250.00 mg/ml, with the lowest minimum inhibitory concentration (MIC) value seen against E. aerogenes and E. faecalis. Out of all the microorganisms tested, B. subtilis was the most susceptible, exhibiting an inhibition zone with a diameter of 17.00 mm (MIC; 31.25 mg/ml). Subsequently, S. aureus and B. cereus have a same minimum inhibitory concentration (MIC) value.

Table 2 demonstrates that the essential oil had significant antibacterial activity against several strains including B. cereus, B. subtilis, S. aureus, S. epidermidis, P. mirabilis, and C. albicans. Out of these strains, the microorganism that was most sensitive was B. subtilis ATCC 6633, with a minimum inhibitory concentration (MIC) value of 15.62 g/ml. This was followed by B. cereus and S. aureus, which had a MIC of 31.25 g/ml.

Based on our comprehensive literature review, there are several papers available that provide evidence of the antibacterial properties of the Salvia genus. Furthermore, our research group has previously documented the antibacterial properties of S. tomentosa, S. cryptantha, and S. multicaulis (Tepe et al., 2004; Tepe et al., 2005). However, we were unable to find any reports specifically addressing the antibacterial characteristics of S. cedronella.

The antiviral efficacy of the plant samples was evaluated in 2 model systems. The replication of two strains of influenza viruses occurred in MDCK cells, whereas the replication of two strains of herpes simplex viruses occurred in MDBK cells (Table 2). The methanol extract of S. cedronella exhibited a potent anti-influenza virus activity, leading to a considerable reduction in the development of both A/Weybridge and A/Aichi strains.

Antiviral activity

This extract also shown antiviral action against herpes viruses.

There is a limited amount of published evidence available on the antiviral properties of Salvia species. Tada et al. (1994) extracted antiviral diterpenes from S. officinalis. In their study, Han and Lee (1999) asserted that the roots of S. miltiorrhiza had antiviral properties. Sivropoulou et al. (1997) demonstrated that the essential oils of S. fruticosa effectively deactivated the herpes simplex virus. The active components in the combined herbal preparation had anti-influenza virus effects and were derived from S. officinalis extracts (Manolova et al., 1995). Additionally, S. officinalis extracts were employed for the treatment of herpes labialis (Saller et al., 2001).

Salvia species have been recognized and utilized as traditional medicines since ancient times. They are known for their antibacterial, antituberculosis, antiviral, cytotoxic, cardiovascular, liver protective, and other properties. Phytochemical investigations have revealed that Salvia species are primarily abundant in diterpenoids (Topcu and Ulubelen, 2007; Ulubelen and Topcu, 1992; Ulubelen et al., 1995; Ulubelen et al., 1992) and triterpenoids (Topcu, 2006; Topcu et al., 2004; Topcu et al., 1994), as well as flavonoids

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(Topcu et al., 1995) and other phenolic compounds (Lu and Foo, 2002).

Phenolics are dietary chemicals that are not necessary for the body's functioning. They have been linked to the prevention of artherosclerosis and cancer. The amount of phenolics in plant extracts was shown to be closely connected to their biological effects (Velioglu et al., 1998). During the progression of our Investigate the antibacterial and antiviral characteristics of S. cedronella essential oils and extracts with different polarities, specifically focusing on an endemic species in Turkey. The study revealed significant antimicrobial and antiviral activity against the tested microorganisms and viruses.

To enhance the data offered here, more investigations are required to provide a more comprehensive understanding of the cytotoxicity and other biological characteristics of the plant species mentioned.

Table 1. Antimicrobial activity of the extracts

Microorganisms	**Samples**	
	D D	**MIC**
B. cereus ATCC 11778	28.00 (OFX)	62.50
B. subtilis ATCC 6633	28.00 (OFX)	125.0 0
E.r aerogenes ATCC 13048	20.00 (NET)	31.25
E.s faecalis ATCC 29212	18.00 (SCF)	31.25
P.s aeruginosa ATCC 27853	22.00 (NET)	15.62
S.s aureus ATCC 25923	22.00 (SCF)	31.25
S. epidermidis ATCC 12228	12.00 (SCF)	15.62
L.a monocytogenes ATCC 19115	12.00 (OFX)	125.0 0
C.a albicans ATCC 90028	28.00 (Amp B)	31.25

Table 2. Antiviral activity of extracts

Samples	**A/Aichi**			
	EC (mg/ml)	**EC50 (mg/m l)**	**SI**	**EC90 (mg/m l)**
Hexane extract	-	> TC50	-	-
Dichloromethane extract	-	> TC50	-	-
Methanol extract	0.55	0.55	6.00	-
Rimantadine	-	0.12	>	-

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hydrochloride		ug/ml	250.00	
	Toxicity to MDBK cells and anti-herpetic virus effect			
Hexane extract	-	> TC50	-	-
Dichloromethane extract	-	> TC50	-	-
Methanol extract	-	0.55	5.5	0.11
BVDU d	1.1ug/ml	1.8 ug/ml	> 60	1.8 ug/ml

CONCLUSION

In vitro tests have been conducted to determine whether or not the various extracts has the capacity to inhibit the growth of bacteria and viruses. The essential oil was subjected to GC-EIMS analysis, which led to the identification of 92 components that were responsible for 96.1% of the oil. 1,8-cineole, a-pinene, caryophyllene oxide, and sabinene were the major components that were present. There was no evidence of antibiotic activity in either the hexane or dichloromethane extracts. There were varied degrees of efficiency shown by the essential oil and the methanolic extract against the bacteria that were tested. Additionally, it has been shown that this extract contains a higher concentration of flavonoids than was previously thought. It was found that there was a positive correlation between the amount of phenolic compounds that were present in the extracts and the potential for biological activity.

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