INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL INVESTIGATION OF ESSENTIAL OILS FROM *Psidium guajava* LEAVES GROWN IN NORTHERN HIMALAYANS

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ARTICLE INFO

Article history
Received 31/05/2023
Available online
26/06/2023

Keywords
Antioxidant,
Antimicrobial,
Phytomedicine,
Herbal Drug.

ABSTRACT

This study was conducted with the intention of determining the total phenolic and flavonoid content (TPC and TFC) of methanolic extract (ME) of P.guajava from the Himalayas region, in addition to analyzing its antibacterial activity (against oral pathogenic bacteria) and its capacity to scavenge free radicals. Substantial action was also achieved against both of the fungal strains that were examined, with Candida albicans demonstrating the highest level of activity. According to the results of the antibacterial activity, the organisms most susceptible to the treatment were Staphylococcus aureus and Escherichia coli. The extract contained a total of 26 components, seven of which were discovered in the extract for the first first time as a result of high-resolution liquid chromatography-mass spectrometry testing.

DOI NO: 10.5281/zenodo.8081295

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*Please cite this article in press as **Mohammad Waseem** et al. Phytochemical Analysis and Antimicrobial Investigation of Essential Oils from Psidium Guajava Leaves Grown in Northern Himalayans. Indo American Journal of Pharmaceutical Research.2023:13(06).*

INTRODUCTION

According to the World Health Organization (WHO), traditional medicines constitute the primary source of medical attention for eighty percent of the world's ever-increasing population. Over the past few decades, there has been a growth in the number of people in industrialized countries who are turning to complementary and alternative medicine (CAM), particularly herbal treatments. Plant remedies have been used to treat a variety of human conditions for a very long time. Plants and the many phytoconstituents they contain continue to be the focus of a significant amount of research despite the fact that they fulfill the requirement for nutrition. In plants, secondary metabolites come in a wide variety of forms, and it's possible that some of these compounds are what contribute to the plants' therapeutic value. Essential oils, terpenoids, glycosides, phenolic compounds, alkaloids, saponins, flavanoids, and tannins are some of the substances that fall under this category. The therapeutic relevance of different plants increases as a result of the increased value of particular chemicals contained inside the plants. In order to prevent infections, alleviate symptoms, and return normalcy to a person's life, various plant parts are being employed. These plant parts are being used since they are inexpensive and have less bad effects. Herbs, herbal materials, herbal preparations, and finished herbal products are all terms that refer to the same thing: medicinal plants and plant components used for therapeutic purposes. There have been a lot of studies conducted to determine the pharmacological action of medicinal plants, and these plants appear to be a potential source for the development of new drugs. This could help to increase the effects of chemical drugs, which could help to reduce the cost of treatment and improve the quality of life for patients. Because of their potential to reduce the effects of oxidative stress and the likelihood of developing degenerative diseases, natural antioxidants such as phenolic compounds are of tremendous benefit to human health. The use of plants as a kind of treatment for illness has a long and illustrious history that can be traced back thousands of years in various countries and probably even to the beginning of human civilization. Since the beginning of time, people have believed that natural medicines, particularly medicinal plants, are the cornerstone of the healing process. Both industrialized countries and developing nations have shown signs of a growing interest in and acceptance of natural medicines over the past few years. Iran is home to a wide variety of plants that have potential uses in medicine. In light of the intricacy of the problem and the negative effects that pharmaceutical treatments can have, the utilization of natural and herbal remedies has been given significant thought. Plant-based medicines are preferred since they have cheaper prices and less negative side effects. In recent years, there has been a meteoric rise in the number of people turning to herbal treatments. In addition, prior to making use of plants or the active components of plants, it is generally suggested that crucial coordination be established and that the necessary scientific proof be obtained.

In recent decades, there has been a growing trend toward favoring the utilization of natural materials over synthetic ones. The amount of time and effort necessary to recycle synthetic materials and goods into something that is less detrimental to the environment is enormous when compared to the time and effort required to recycle natural ingredients. Secondary metabolites are produced by every plant in existence, and it's possible that this process is what gives plants their potential therapeutic value. Tannins and phenolic compounds are two examples of metabolites. Essential oils and terpenoids, glycosides and terpenes, and even alkaloids are all examples of metabolites. The therapeutic relevance of different plants increases as a result of the increased value of particular chemicals contained inside the plants. It has been thoroughly explored that the pharmacology of medicinal plants, which makes them a valuable resource for the development of innovative medications and possibly boosting the efficacy of existing chemical medicines, which might cut healthcare expenses without affecting the quality of care. If you minimize the amount of oxidative stress in your body and prevent macromolecules from being oxidized, you'll begin to understand why natural antioxidants like phenolic compounds are so beneficial to human health.

Diabetes, viral problems, cancer, and atherosclerosis are just few of the many diseases that can be treated thanks to the information that can be learned through studying medicinal plants. There are many more diseases that can be addressed. It's possible that medicines made with natural chemicals, as opposed to synthetic ones, will be more effective while also causing less negative side effects. In conventional medicine, the use of herbs places a strong emphasis on hypoglycemic components or other disease-related adverse effects.

The Psidium guajava tree, often known as the royal walnut tree, is a member of the family Juglandaceae and has therapeutic purposes. Despite the fact that it may have potential therapeutic effects, walnut is undervalued, much like a large number of other herbal remedies utilized in traditional medicine. The roots of the walnut tree are used to treat diabetes, while the flowers of the walnut tree are used to treat malaria and rheumatic discomfort. Walnut leaves are a traditional remedy for a variety of ailments, including rheumatic pains, fever, diabetes, and skin conditions. This herb, when used in the traditional cuisine and medicine of the Iranian culture, is said to be effective against a broad variety of diseases. Walnut leaves are a common component in traditional Chinese medicine for the treatment of diabetes because of their ability to bring blood sugar levels down. Due to the discovery of chemicals in walnut leaves that have been found to have therapeutic effects on human health, walnut leaves have been extensively used in traditional medicine for the treatment of venous insufficiency and hemorroides. Others use them to treat diarrhea and parasites, while others take them to purify the blood. All of these uses are beneficial. According to the findings, the pharmacological effects of walnut have been the subject of a significant amount of research over the past two decades. The blood sugar levels of diabetic animals may be lowered by consuming any component of this plant, including the leaves, the bark, and the fruits. Lower cholesterol levels were seen in streptozotocin-induced diabetic rats that had been given walnut leaf extract, proving its efficacy. Recent research has also linked this herb's use to analgesic and antibacterial benefits.

MATERIAL & METHODS

Reagents & Instrument

Sodium nitrite, aluminum chloride, gallic acid, and quercitin were acquired from the Sigma-Aldrich Chemical Corporation, as well as 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulonic acid) (ABTS), and ascorbic acid (ABA) (St. Louis, Mo., USA). Measurement-grade organic solvents and an analytical-grade multimode reader (both from Tecan, Austria) were used to test for antioxidant and antibacterial properties (Merck, Darmstadt, Germany). Polyphenolics were analyzed and identified using an Agilent Technologies UHPLC-PDA-Detector Mass spectrometer (HR-LCMS 1290 Infinity ULC System, 1260 infinity Nano HPLC with Chipcube, 6550 iFunnel QTOFs), and FT-IR spectra were recorded using a Spectrum RX1 FT-IR spectrophotometer (Perkin Elmer, USA).

Extracts preparation

In-vitro Antioxidant Activity

ABTS Scavenging Activity

According to the protocol laid forth by Re et al., the pollen extract was put through a battery of tests to evaluate if it possesses the ability to scavenge ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals. Following the addition of the extract to the ABTS solution, we waited for a period of six minutes. It was determined that the absorbance occurred at a wavelength of 734 nm. The units of analysis were millimoles of trolox equivalents per gram of dry weight, abbreviated as mmol TE/g dw.

FRAP Assay

Mix 10mM Tripyridyltriazine TPTZ in 40mM HCL, 20mM FeCl3. 6H2O, and 300mM Acetate Buffer together in a mixing container to make the FRAP reagent. In brief, 50 ml of a herbal preparation varying in concentration from 200 µg to 1000 µg was mixed with 1.5 ml of freshly manufactured and pre warmed FRAP reagent and the mixture was incubated for 10 minutes at 37 °C. This was done so that the FRAP activity of the herbal preparation could be calculated. The blank used in this experiment was distilled water, and its absorption was compared to that of a blue complex. The bibliography had entries for both BHT and tocopherol. The peak absorption was found to be at a 593 nm wavelength. The conversion of ferric to ferrous ions was measured to occur at a rate of mMol FRAP. Each experiment's IC 50 value was calculated, and statistical significance was defined as a p value of less than $p < 0.001$. The result was presented as the mean SD of three separate experiments expressing the percentage of inhibition (% Inhibition).

DPPH Assay

Antioxidants inhibit lipid oxidation in a variety of different ways, one of which is by scavenging for and neutralizing free radicals. The use of DPPH as a tool for determining whether or not a substance may scavenge free radicals has become widespread in recent years. It has a color similar to that of the color purple, and it has a pronounced absorption at a wavelength of 517 nm. Moreover, it is a free radical that is stable but dissolves in methanol. Antioxidants lessen the absorbance of the purple DPPH assay solution by giving protons to it, which then causes the solution to take on a light yellow color. Examining the absorbance drop allows for a proxy measurement of the efficiency of radical scavenging. The results of this test were reported as a percentage, which represented the ratio between the absorbance of DPPH radical solution at 517 nm and the absorbance of DPPH radical solution at the same wavelength after being exposed to extract. The absorbance of DPPH radical solution at 517 nm was divided by the absorbance of DPPH radical solution at the same wavelength to arrive at this value. The ability of extracted samples to scavenge DPPH radicals is represented in Figure 1 alongside that of BHA and TBHQ, which serve as standard compounds. Also depicted in this figure is the ability of standard compounds BHA and TBHQ to do the same thing. Above a certain level of phenolics, the concentration dependence of the scavenging activity in the sample curve leveled off, which indicated that additional increases in concentration did not result in increased antioxidant activity. Since about 95.6% of DPPH was scavenged at a concentration of 100 ppm, whereas only 91.6% of radicals were scavenged at a concentration of 50 ppm, this suggests that TBHQ is an efficient radical-scavenger for DPPH. At doses of 200 and 400 ppm, respectively, both BHA and the sample extract were shown to be equally effective at scavenging radicals. At doses of 400, 200, and 50 ppm, respectively, the radical scavenging activity of the sample, BHA, and TBHQ were all comparable. Samples, BHA, and TBHQ EC_{50} values were all calculated using the DPPH assay (table 2). When antioxidant activity increases, the EC_{50} value drops. Consistent with the findings of the previous study by Oliveria et al (2008). In both studies, the EC50 for the walnut green husk extract was found to be below 1 mg/mL, although our results revealed even lower concentrations (stronger scavenging effect).

Antimicrobial Activity

In order to determine whether or not plant extract is effective against bacteria, a well diffusion assay was performed [10]. The turbidity of the subcultured bacteria was brought up to the criterion of 0.5 McFarland, which is equivalent to 1.5×10^8 cells/ml. In order to inoculate the Mueller-Hinton agar with the test microorganisms, the spread plate technique was utilized. After allowing the wells to sit for thirty minutes at room temperature, a borer was used to make holes in them. After adding dimethyl sulfoxide (DMSO) to the dried extract, 50 µl of the extract was afterwards distributed evenly across the wells. In order to establish the standard, the antibiotic chloramphenicol (HiMedia) was utilized. We also used DMSO as a negative control in one of our wells so that we could be absolutely certain that it did not possess any antibacterial properties. The size of the inhibition zone was measured after 24 hours of incubation and compared to the inhibition zone produced by a reference antibiotic.

Statistical Analysis

Three replicates of each test were conducted, and the results were reported as a mean ±standard deviation. We did an ANOVA on the data in SPSS and then used Duncan's multiple range tests at the ($p< 0.05$) level of significance to draw conclusions.

RESULT & DISCUSSION

Using high-resolution liquid chromatography mass spectrometry, we analyzed the methanolic extract of J. regia (MEJR) for its antioxidant, extraction yield (percent), total phenolic compound (TPC), and total phenolic compound (TFC) content and antibacterial activity (HR-LCMS). Retention periods, absorbance spectra, and mass spectrometry data all show that MEJR contains 26 distinct bioactive compounds. The masses of the compounds, as well as their chemical names and retention durations, are included in Table 1. Many of these compounds, including the amino acid Arginine, which may be found in *J. regia* L. nuts, have been extracted from various parts of the plant growing in a variety of ecological environments. It has been hypothesized that the 11-amino-undecanoic acid (ursolic acid) found in the green husks of J. regia grown in China can also be found in the nuts produced by six distinct J. regia Cultivars grown in Portugal. Among the leaves, green husk, stem bark, and nuts of *J. regia*, researchers found 2,2-Dimethyl-3-Oxo-Butyric Acid 2-Trimethylsilanyl; Quercetin-3-O-glucuronide; Hexanoic acid, trimethylsilyl ester; Oleic acid; n-Hexadecanoic acid; and 1,2-Benzene dicarboxylic acid, bis-(2-methyl propyl) According to our best knowledge, *J. regia* is the first plant to have the chemical components of its tissues characterized. Elephantopin, gamma-L-glutamyl-cysteine, artemisinin, madecassic acid, dihydromyricetin, swietenine, securinine, and sphinganine are some of the substances that fall under this category. Variations in phytochemistry may be the result of a number of environmental conditions, including but not limited to seasonal shifts, variations in habitat, and other ecological factors.

Table 1: The methanolic bark extract of P. Guajava has undergone preliminary phytochemical screening.

Phytoconstituents	ME of Guajava leaves
Flavonoids	++
Phenols	++
Alkaloids	_ _
Terpenoids	++
Cardiac glycosides	++
Saponins	++
Amino acids	_ _
Carbohydrates	++
Tannins	++
Fixed oils and fats	_ _
Steroids	_ _
Phlobatannins	+ +

Antioxidant activity

As can seen in Table 2, the DPPH, ABTS, and FRAP tests were utilized in order to evaluate the degree to which three different extracts of the Leaves P. guajava differ in their levels of antioxidant activity. The results of the DPPH, ABTS, and FRAP assays, all of which are dependent on electron transfer between the sample and the reagent radical, can be determined by the use of spectrophotometric measurement of color changes. In the DPPH, BTS, and FRAP experiments, all three J. regia extracts demonstrated considerable free radical scavenging activity, which was measured in comparison to the high antioxidant effect exhibited by ascorbic acid. EEJR had IC50 values of 75.17±4.43 and 63.40±5.73 for DPPH and ABTS, respectively; while, AEJR had a FRAP value of 54.35±3.12 µmol/L FeSO4/mg extract. The IC50 values for MEJR using DPPH and ABTS were 66.80±2.13 and 43.60±4.83µmol/L, respectively. We discover, through the use of DPPH, ABTS, and FRAP assays, that the ability of various extracts to scavenge free radicals is comparable to that of the leaves, bark, stem, and nuts of J. regia.

Table: 2 Antioxidants Activity.

IC_{50}					Contents (mg/g)	
Sample	Yield(%)	DPPH	ABSTS	FRP	TFC	TPC
MEJR	12.3	66.80±2.13	53.9±3.01	43.60±4.89	144.65±5.09	129.76±3.11
EEJR	8.8	75.17±4.43	63.40±5.73	54.35±3.12	137.41±4.91	124.12±2.45
AEJR	7.9	76.76±4.78	64.30±5.89	52.70±2.47	131.79±4.78	12.2±2.90

Antimicrobial Activity

Those aromatic and oily liquids that are extracted from plants and are sometimes referred to as volatile oils or ether oils. The majority of their chemical compositions are comprised of terpenoids, acids, alcohols, aldehydes, ketones, acyclic esters, lactones, and, less frequently, nitrogenous and sulfur compounds, coumarins, and phenylpropanoid homologs. In Table 3, we identify the components of the essential oil that was extracted from the leaves of the Psidium guajava tree, and the gas chromatogram of the essential oil can be found depicted in Figure 1 of our research.

Table: 3 Essential oil composition of *Psidium guajava.*

RT (min)	Component	Quantity(%)
20.088	octadecanal	3.28
21.622	2-dodecene	4.84
25.185	Hexadec-7-en-16-olide	0.74
25.844	Methanone	2.58
27.029	5-octadecene	6.22
27.177	Oxalic acid	1.08
27.985	Octadecane	0.94
28.607	tetradecanoic acid	1.22
29.659	9-octadecene	17.71
31.089	cycloheptadecanone	0.16
32.326	Hexadecanoic acid	11.32
33.837	Bicyclo[13.1.0]hexadecane-2-one	0.44
34.785	8-hydroxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-cisdecalin	2.34
35.533	Octadecanoic acid	6.96
36.807	Cembrene	9.49

The essential oils that were isolated from Psidium guajava leaves included the highest levels of 9-octadecene (17.71%), hexadecanoic acid (11.32%), and cembrene (9.49%). (Table 1). Analyses of walnut leaves, looking for the presence of the essential oil that walnuts are known to contain, have been the subject of a number of research. In Mateur, which is located in northern Tunisia, Bou- Abdallah et al. utilized a method known as hydrodistillation in order to extract essential oils from walnut leaves (2016). According to the findings, the concentration of caryophyllene oxide ranged from 16% to 27.4%, and the concentration of -caryophyllene ranged from 4% to 22.5% [4].

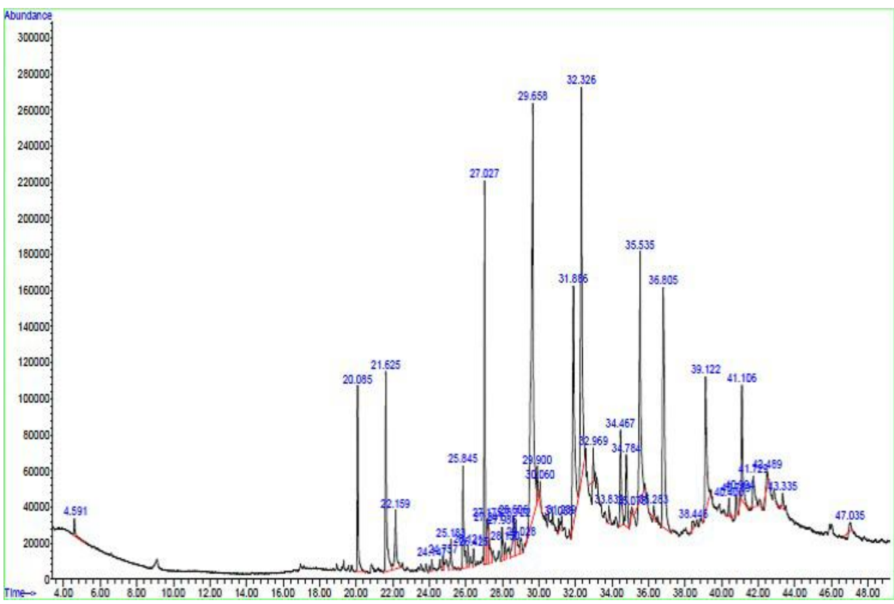

Fig: 1 The gas-chromatogram of the essential oil of *Psidium guajava.*

Rather et al. (2012) were able to successfully extract essential oils from walnut leaves that were cultivated in the Indian state of Uttarakhand by using hydrodistillation. [20] They discovered high concentrations of -pinene (15.1%), -pinene (30.5%), -caryophyllene (15.5%), germacrene D (14.4%), and limonene (3.6%). Antibiotics are the one family of pharmaceuticals that stand out as being particularly useful in the fight against the transmission of microorganisms. These health benefits, however, have been put in jeopardy over the past few years as antibiotics that are commonly used have shown to be less successful against certain infections. This is not only due to the severe reactions that antibiotics provoke, but also due to the rise of bacteria that are resistant to drugs. Both disease prevention and disease treatment in humans can benefit greatly from the use of herbal medicines. Herbal remedies play a significant role in both. The results of the antibacterial activities are presented in Table 2. Both S. aureus subsp. aureus and P. vulgaris were shown to be more sensitive to the antibiotic than any of the other bacterial species that were put to the test. The breadth of the growth-inhibition zone that was measured was somewhere between 6 and 17 millimeters. Values with a diameter of less than 8 mm were deemed inactive against microbes [3].

Table 2. Inhibition zones of the essential oils of P. guajava and antibiotics (mm).

Bacteria	Essential oil of *P.guajava*	DMSO	Tetracycline	Gentamycin
S.enterica serovar typhimirium ATCC 14028(gram-)	7	-	15	17
Y. pseudotuberculosis ATCC 911(gram-)	6	-	-	20
E. aerogenes CCM 2531(gram-)	13	-	11	15
B. subtilis IMG 22(gram+)	9	-	11	16
S. aureus subsp.aureus ATCC 25923(gram+)	17	-	18	19
P. vulgaris FMC1(gram-)	17	-	11	15
B. cereus 702 ROMA (gram+)	14	-	10	17

The highest results were obtained against S. aureus subsp. aureus (17 mm) and P. vulgaris (17 mm), whereas the worst results were obtained against Y. pseudotuberculosis (17 mm) (6 mm). When the susceptibility of various bacterial pathogens was compared, it was found that gram-positive bacterial strains were, on average, more susceptible than gram-negative bacterial strains. This was discovered while comparing the susceptibility of various bacterial pathogens. This is because the cell walls of gram-positive and gram-negative bacteria are formed very differently from one another. The antibacterial agent DMSO (dimethyl sulfoxide), which served as a negative control, was found to be ineffective.

CONCLUSION

It was revealed that 9-octadecene accounts for 17.71 percent of the composition of the essential oil that is distilled from the leaves of *Psidium guajava*, followed by hexadecanoic acid (11.32%), and cembrene (9.49 percent). The results of this study offer compelling evidence that the essential oil of J. regia possesses the potential to be utilized as an effective antibacterial agent against the oral bacteria that are responsible for a wide variety of oral illnesses. The findings of this study were published in the journal Oral Microbiology and Immunology. Mouthwash made from medicinal plants is one type of effective home care medicine that may be used to maintain and even increase one's level of oral cleanliness. It has been suggested that the usage of *P.gaujava* oil, which is a rich source of therapeutic components such as phenols and flavonoids, could be effective in the prevention of diseases brought on by free radicals.

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