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Phytotherapy-Based Insights: Antidiabetic, Antioxidant , Antimicrobial and Anticancer Activities of *Psidium Guajava* L. and *Withania coagulans* with Gas Chromatography-Mass Spectrometry Profiling

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This study explores the phytotherapeutic potential of *Psidium guajava* L. (Leaves) and *Withania coagulans* (Berries) through an in-depth assessment of the antidiabetic, antioxidant, antimicrobial, and anticancer activities of four extracts prepared using the maceration method with methanol and water. The presence of flavonoids, tannins, terpenoids, and saponins in all four extracts was revealed using Phytochemical screening. The antidiabetic activity was assessed across all extracts, with the methanolic extract of *Psidium guajava* L. exhibiting the strongest effect, followed by both extracts of *Withania coagulans*, which demonstrated notable efficacy. The antioxidant activity was highest in the *Psidium guajava* L methanolic extract, compared to the other three extracts. Antimicrobial activity against *Staphylococcus aureus* was evaluated for all extracts, with the methanolic extract of *Withania coagulans* showing the most significant inhibitory effect. In anticancer assays targeting lung cancer cells, the *Withania coagulans* extracts displayed promising potential, particularly the methanolic extract. Given the strong bioactivity observed in the methanolic extracts of both plants, GC-MS analysis was carried out to pinpoint the bioactive compounds responsible for their therapeutic effects. The study sheds light on the potential of *Psidium guajava* L. and *Withania coagulans* as rich sources of bioactive compounds, offering promising applications in phytotherapy for managing diabetes, oxidative stress, microbial infections, and cancer.

Keywords: Anticancer activity; Antidiabetic activity; Antimicrobial activity; Antioxidant activity; GC-MS; Phytotherapy; *Psidium guajava* L.; *Withania coagulans*.

Major health concerns such as diabetes, lung cancer, microbial infections, and oxidative stress are prevalent worldwide, affecting millions of individuals each year. Characterized by elevated and persistently high blood sugar levels, diabetes is a chronic metabolic disease that has spread worldwide at epidemic levels.

Diabetes mellitus is a long-term disorder affecting glucose metabolism, with significant clinical repercussions. Diabetes can lead to microvascular complications like retinopathy, nephropathy, and neuropathy, as well as macrovascular issues such as ischemic heart disease, stroke, and peripheral vascular disease. In

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recent years, its prevalence has grown significantly, primarily due to the worldwide increase in obesity rates. The condition contributes to early morbidity and mortality, decreased life expectancy, and substantial financial burdens for patients, caregivers, and healthcare systems, making it a critical public health issue.[1]

Lung cancer poses a significant global health challenge and remains the leading cause of cancer-related mortality worldwide. Lung cancer is broadly classified into two main types: non-small cell lung cancer (NSCLC), accounting for approximately 85% of cases, and small cell lung cancer (SCLC), which is less common but more aggressive. It remains a major global health concern, imposing substantial challenges on both patients and healthcare systems. According to GLOBOCAN 2022, lung cancer accounted for 2,480,675 new cases worldwide. However, its mortality rate is particularly alarming, with 1,817,469 deaths recorded in the same year, the highest among all cancers. The treatment for lung cancer involves surgery, chemotherapy, radiation, targeted therapy, and immunotherapy, but often these approaches cause severe side effects such as fatigue, immunosuppression, organ damage, and inflammation, impacting patients' quality of life .[2]

When there is an imbalance between elevated levels of reactive oxygen species (ROS) and insufficient antioxidant defenses, Oxidative stress occurs. While excessive oxidative stress can damage cellular structures and lead to tissue destruction, ROS are essential for normal cellular functions, including mitochondrial energy production. This imbalance has been implicated in physiological processes like aging and exercise, as well as cancer-like pathological conditions, neurodegenerative disorders, cardiovascular diseases, diabetes, inflammatory conditions, and toxic exposures.[3]

Staphylococcus aureus is a resilient and opportunistic pathogen that primarily colonizes the anterior nares, serving as its main reservoir. It is responsible for a broad spectrum of infections ranging from superficial skin conditions such as boils, impetigo, and furuncles, to severe systemic diseases like septicemia, toxic shock syndrome (TSS), and endocarditis. Additionally, S. aureus is a significant cause of infections in surgical wounds, trauma injuries, and indwelling medical devices. It also leads to complications such as pneumonia, osteomyelitis, meningitis, and mastitis, posing serious health risks.[4]

Phytotherapy, the science of using plant-based medicines for therapeutic purposes, has been in practice since the earliest of times and continues to play a significant role in present-day healthcare. With roots in traditional systems such as Ayurveda, Traditional Chinese Medicine, and Unani, phytotherapy leverages the bioactive compounds found in plants to intercept and cure a wide range of diseases. This field has gained renewed interest in recent decades as scientists and healthcare professionals seek alternative and complementary approaches to conventional medicine.[5]

The appeal of phytotherapy lies in its multidimensional benefits. Beyond providing targeted treatment for conditions such as diabetes, cancer, and cardiovascular diseases, phytotherapy contributes to holistic well-being. Plants contain an abundance of bioactive compounds including alkaloids, flavonoids, terpenoids, and phenolics, which exhibit antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties. These attributes make phytotherapy a promising avenue for addressing chronic diseases and promoting long-term health. [6]

This research seeks to explore the diverse therapeutic potential of phytotherapy by assessing the antimicrobial, antidiabetic, anticancer, and antioxidant properties of specific plant extracts of *Psidium guajava* L. and *Withania coagula*ns. It emphasizes the bioactive compounds within these extracts, providing an in-depth understanding of the significance of plant-based treatments in contemporary healthcare.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Psidium guajava* L. were gathered from the Anjar region of Kutch. Berries of *Withania coagula*ns were purchased from the ayurvedic stores of Gandhinagar city in Gujarat.

Preparation of Plant Material

The plant material was processed through several essential steps to preserve bioactive compounds and ensure high quality. Freshly collected materials were thoroughly cleaned with

water to remove dirt, dust, and impurities. To prevent spoilage and retain beneficial components, plant materials were dried naturally in the shade for 7 to 15 days, a process that protects their sensory attributes and bioactive properties. Once dried, the plant materials were ground into a fine powder to maximize the surface area for efficient extraction of active ingredients. The resulting powder was stored at a lower temperature to maintain its integrity for subsequent use.[7]

Preparation of Plant Crude Extracts

The crude extracts were prepared using the maceration method, a simple yet effective technique to extract bioactive compounds. Two solvent systems were employed for this process: one using water and the other methanol. A plant powder-to-solvent ratio of 1:10 was maintained, meaning 30 grams of powdered plant material was mixed with 300 milliliters of each solvent.

The mixtures were kept at room temperature for one week, during which they were periodically agitated to enhance the extraction process. This gentle stirring ensured maximum interaction between the plant material and the solvents, aiding the release of active compounds. After a week, the mixtures were filtered to remove solid residues. The filtrates were then evaporated at specific temperatures appropriate for each solvent to concentrate them into semisolid products.[8]

Phytochemical screening

Phytochemicals are naturally occurring, non-nutritive compounds found in plants that contribute to their medicinal properties and offer disease-preventive benefits.

In this study, phytochemical screening was carried out on extracts of *Psidium guajava* L. leaves and *Withania coagulans* berries, using water and methanol as solvent systems. The screening was performed following the methods outlined by Ayoola et al. (2008). The plant extracts were tested for the presence of key bioactive compounds, including terpenoids, flavonoids, saponins, tannins, Alkaloids and Anthraquinones, which are recognized for their therapeutic and disease-preventive properties .[5,9]

Test for Terpenoids (Salkowski Test)

To detect terpenoids, 0.5 grams of each plant extract were combined with 2 milliliters of chloroform in a test tube. Subsequently, 3 milliliters of concentrated sulfuric acid were gently added to form a separate layer. The appearance of a reddish-brown coloration at the interface of the layers confirmed the presence of terpenoids, which are bioactive compounds with notable medicinal properties.

Test for Flavonoids

To test for flavonoids, 0.5 milliliters of each plant extract filtrate were combined with 5 milliliters of a dilute ammonia solution. Subsequently, 1 milliliter of concentrated sulfuric acid was added cautiously. The presence of flavonoids was indicated by the formation of a yellow coloration, which gradually faded over time, providing further confirmation.

Test for Saponins

To test for saponins, 0.5 grams of each plant extract were combined with 5 milliliters of distilled water in a test tube. The mixture was vigorously shaken and observed for the development of a stable, persistent froth. To confirm the result, 3–4 drops of olive oil were added, and the solution was shaken again. The formation of an emulsion signified the presence of saponins.

Test for Tannins

To test for tannins, 0.5 grams of each plant extract were boiled in 10 milliliters of water and subsequently filtered. A few drops of 0.1% ferric chloride solution were added to the filtrate. The presence of tannins was indicated by the development of a brownish-green or blue-black coloration.

Test for Alkaloids

0.2 grams of plant extract were dissolved in 10 milliliters of acid alcohol, boiled, and filtered. To 5 milliliters of the filtrate, 2 milliliters of dilute ammonia were added, followed by 5 milliliters of chloroform. The mixture was shaken gently, and 10 milliliters of acetic acid were added. The solution was divided, and the upper layer was discarded. Draggendorff's reagent was added to the remaining layer. The presence of alkaloids is indicated by the formation of a reddish-brown precipitate

Test for Anthraquinones

To test for alkaloids, 0.2 grams of each plant extract were boiled with 10 milliliters of sulfuric acid, then shaken with 5 milliliters of chloroform. The chloroform layer was separated

into a new test tube, and 1 milliliter of dilute ammonia was added. Any color change in the solution indicated the presence of Anthraquinones.

In-vitro Anti-diabetic Activity Assay

The anti-diabetic potential of various extracts from *Psidium guajava* L. leaves and *Withania coagulans* berries was assessed in vitro using the method reported by Prabhakar et al. (2013).[10,11] The activity was deduced by measuring the inhibitory effect on the enzyme á-amylase, which breaks down starch into glucose.

The assay was conducted using concentrations of 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL for each plant extract.

In the assay, 1 milliliter of the prepared plant extract was mixed with 1 milliliter of á-amylase enzyme solution in a test tube and incubated for 10 minutes at 37°C. Next, 1 milliliter of 1% starch solution was added, and the mixture was incubated for another 15 minutes at 37°C. The reaction was terminated by adding 2 milliliters of DNSA reagent, followed by a 5-minute incubation in a boiling water bath. After cooling to room temperature, the absorbance was measured at 546 nm using a spectrophotometer. The control, which did not contain the extract, represented 100% enzyme activity. The percentage inhibition of á-amylase activity was calculated using the formula:

% inhibition of á-amylase = (Enzyme activity of control - Enzyme activity of extract) / Enzyme activity of control * 100

Anti-oxidant activity

The antioxidant activity of the plant extracts of *Psidium guajava* L. leaves and *Withania coagulans* berries were evaluated using the DPPH free radical scavenging assay. Extracts were prepared at concentrations of like 10 mg/ml, 20 mg/ mL, 30 mg/ mL, 40mg / mL, and 50mg / mL. for each plant. A 0.004% DPPH solution in methanol served as the reagent, while using ascorbic acid as the positive control. 1ml of DPPH solution was mixed in each sample, vortexed, and incubated in a dark condition at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer. Controls included a blank (80% methanol) and a negative control (DPPH solution with methanol only). The experiment was conducted in triplicate. A lower absorbance of the mixture indicated greater radical scavenging activity, which was calculated using the formula:

$$\text{DPPH scavenging effect (\%)} = \{(Ao - A) / Ao\} \times 100$$

Where, Ao represents the absorbance of the negative control, and A is the absorbance with the extract. Results were articulated as IC50 values and percentages of DPPH scavenging activity, following the procedure given by Mahdi-Pour et al. (2012).[12]

Antimicrobial Activity against *Staphylococcus aureus*

The antimicrobial activity of the plant extracts was evaluated using the agar well diffusion method on nutrient agar plates. *Staphylococcus aureus* was used as the test organism. Initially, the bacterial culture was revived by inoculating it into broth media and incubating it at 37°C for 24 hours.

To prepare the plates, *S. aureus* culture was mixed directly with molten nutrient agar and poured into sterile Petri dishes, allowing the media to solidify. Different concentration of the plant extract (10 mg/ml,20 mg/ml,30 mg/ml,40 mg/mL, 45mg/mL, and 50 mg/mL) were added to the wells. For the negative control, DMSO was used, along with an antibiotic (Amikacin) as the positive control. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around the wells were measured in millimeters to assess the antimicrobial activity.[13]

Anti-cancer activity

The anticancer potential of the plant extracts was assessed using the MTT assay on human lung carcinoma epithelial cells (A549), sourced from the American Type Culture Collection (ATCC). The A549 cells were cultured in the appropriate growth medium. The plant extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted to achieve concentrations of 200 ìg/mL, 400 ìg/mL, 600 ìg/mL, and 800 ìg/mL. The cells were exposed to these concentrations for 24 hours, with untreated cells serving as the control group.[14]

GC-MS

In our study, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed

using a Shimadzu GCMS-TQ8040 NX system, equipped with an Auto Injector (AOC-20i Plus) and an Auto Sampler (AOC-20s Plus). The analytical column employed was 30 meters in length with a diameter of 0.25 mm. The GC inlet temperature was set to 250°C, utilizing a split injection mode with a split ratio of 10.0. Helium served as the carrier gas, with the control mode adjusted for linear velocity. The column oven temperature commenced at 50°C, with a purge flow rate of 5.0 mL/min. A 1 ìL sample injection volume was used. The ion source temperature in the mass spectrometry (MS) section was set to 230°C, and the interface temperature was maintained at 250°C. The detector gain mode was adjusted based on the tuning result, with a gain value of 0.89 kV + 0.20 kV. The identification of bioactive compounds was achieved by correlating their peak retention time, peak area (%), height (%), and mass spectral fragmentation patterns with those documented in the National Institute of Standards and Technology (NIST) library for known compounds. Utilizing NIST library standards enhances the reliability and accuracy of compound identification, thereby strengthening the robustness of the experimental findings.[15]

RESULTS

Phytochemical Screening

The phytochemical screening data presented in Table 1 highlights a diverse distribution of bioactive compounds in water and methanol extracts of *Withania coagulans* berries and *Psidium guajava* L. leaves, emphasizing their therapeutic potential. Triterpenoids are highly concentrated in the water extract of *Withania coagulans* (PH) and moderately present in both extracts of *Psidium guajava* L. Flavonoids, known for their antioxidant properties, are found in very high concentrations in

Table 1. Phytochemical Analysis of Plant Extracts

Phytochemical	PH	PM	GH	GM
Terpenoids	+++	+	++	++
Flavonoids	++	+	+++	++++
Saponins	+++	+	++	+
Tannins	+++	+	++	+++
Anthraquinone	++	+	+++	++++
Alkaloids	+	+++	++	+++

("+" denotes that the phytochemical is present in trace amounts; "++" indicates a moderate presence; "+++" reflects a high concentration of the compound; "++++" reflects very high concentration of the phytochemical)

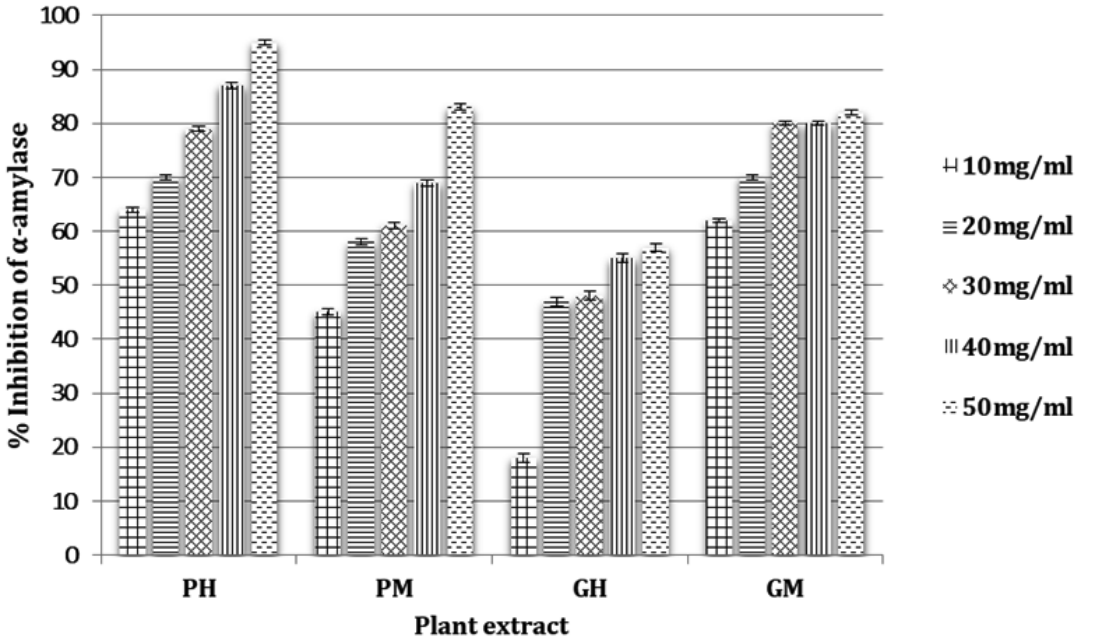

Fig. 1. Anti-diabetic activity of plant extracts

the methanol extract of *Psidium guajava* L. (GM) and moderately to highly present in other extracts. Saponins are abundant in the water extract of *Withania coagulans*, while tannins are significantly present in the water extracts of both plants and in the methanol extract of *Psidium guajava* L.. Anthraquinones are particularly concentrated in the methanol extract of *Psidium guajava* L., while alkaloids are abundant in both methanol extracts. These findings in Table 1 underscore the medicinal relevance of these plant extracts, with the methanol extract of *Psidium guajava* L. showing especially high levels of key phytochemicals.

Anti-diabetic Activity

Figure 1 illustrates the results of the anti-diabetic test performed using the á-amylase inhibition technique. The test measured the inhibitory effect of various plant extracts on á-amylase activity, an enzyme involved in starch breakdown to glucose. The concentrations of the plant extracts ranged from 10 to 50 mg/ml. The data indicate a dose-dependent increase in á-amylase inhibition, with higher concentrations demonstrating more significant inhibitory effects. Extracts at higher concentrations exhibited greater inhibition, showing potential as natural anti-diabetic agents.

These results align with findings from studies on other plant extracts with similar á-amylase inhibitory properties, underscoring the importance of these compounds in controlling postprandial blood glucose levels. The percentage

Table 2. Anti-diabetic activity of different extracts by α-amylase inhibition method

Concentration (mg/ml)	% Inhibition of α-amylase			
	PH	PM	GH	GM
10	60.00 ± 0.45	40.00 ± 0.35	30.00 ± 0.35	50.00 ± 0.40
20	61.00 ± 0.40	45.00 ± 0.40	35.00 ± 0.40	55.00 ± 0.45
30	68.00 ± 0.50	55.00 ± 0.45	48.00 ± 0.45	63.00 ± 0.50
40	79.00 ± 0.60	65.00 ± 0.50	58.00 ± 0.50	74.00 ± 0.55
50	83.00 ± 0.55	72.00 ± 0.60	72.00 ± 0.55	80.00 ± 0.60

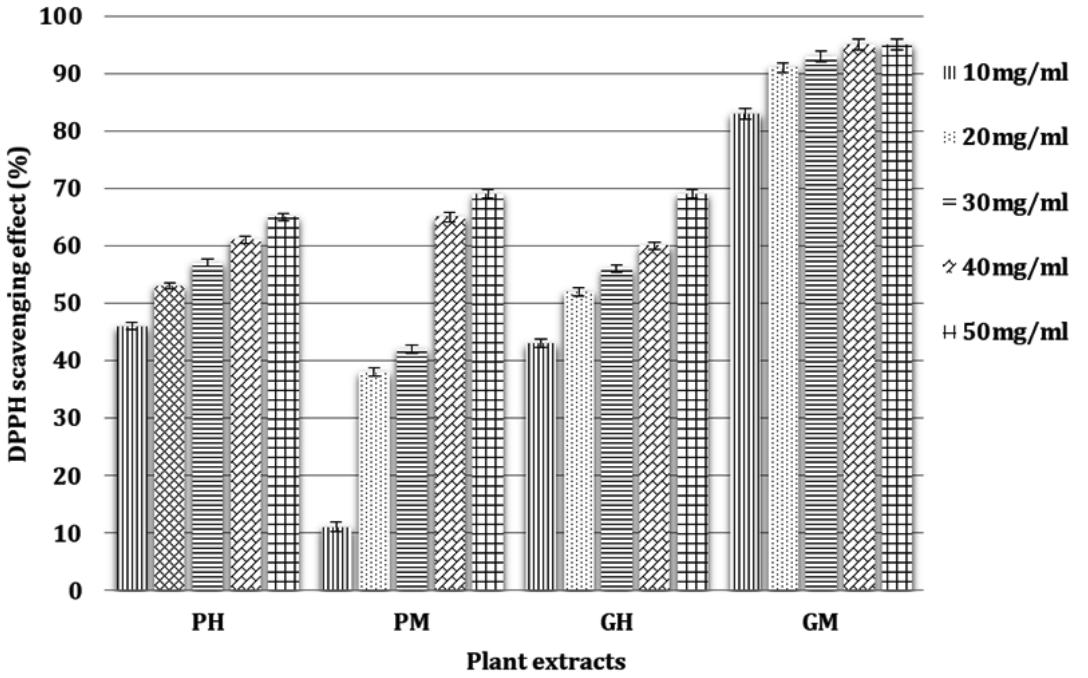

Fig. 2. Antioxidant activity of Plant extracts

reduction in á-amylase activity by four plant extracts (PH, PM, GM, and GH), along with the corresponding error margins is displayed in Table 2. PH showed the highest inhibition, while GH exhibited the lowest.

Anti-oxidant Activity

Table 3 presents the approximate values and associated errors for different concentrations of plant extracts, highlighting the activity levels of water and methanol extracts of *Withania coagulans*

Table 3. DPPH inhibition percentage of different extracts

Sample	10mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml
PH	46± 0.3	53 ± 1.0	57 ± 0.1	61 ± 0.3	63 ± 0.2
PM	11± 0.5	38 ± 0.4	42 ± 0.2	65 ± 0.6	69 ± 0.4
GH	43 ± 0.8	52 ± 0.7	56 ± 0.5	66 ± 0.4	69 ± 0.7
GM	83 ± .05	91 ± 0.3	93 ± 0.3	95 ± 0.4	95 ± 0.3

Table 4. Antimicrobial activity of different extracts *against Staphylococcus aureus*

	Inhibition zone (diameter in mm)			
Concentration (mg /ml)	PH	PM	GH	GM
10	11± 0.2	15± 0.3	08± 0.4	12± 0.3
20	12± 0.4	17± 0.2	09± 0.5	14± 0.5
30	13± 0.3	20± 0.4	10± 0.3	18± 0.6
40	15± 0.5	30± 0.6	11± 0.5	19± 0.4
45	15± 0.7	32± 0.7	12± 0.7	20± 0.5
50	16± 0.5	35± 0.6	12± 0.2	21± 0.3

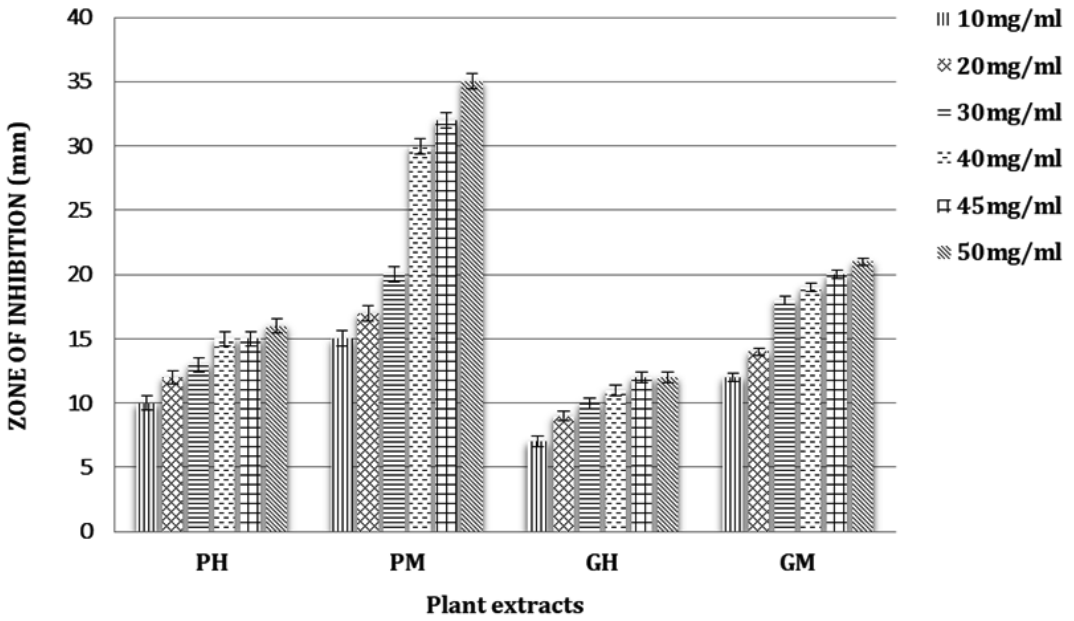

Fig. 3. Antimicrobial activity of Plant extracts

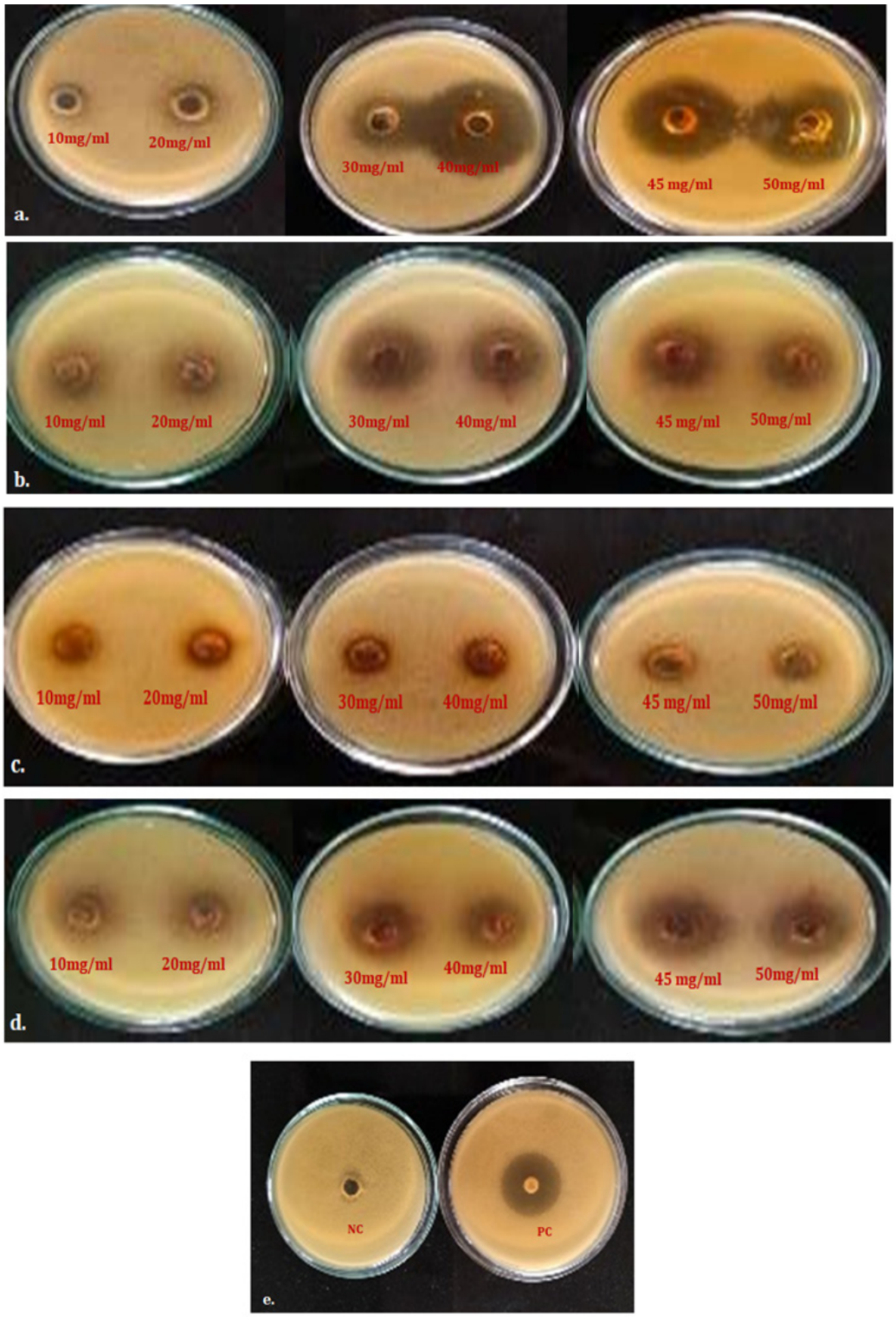

Fig. 4. Anti-microbial activity of plant extracts at different concentrations a: for methanolic extract of *Withania coagulans* berries, b: for water extract of *Withania coagulans* berries, c: for water extract of *Psidium guajava* L. leaves, d: methanolic extract of *Psidium guajava* L. leaves, e: Control

Table 5. Cytotoxic effect of plant extracts at different concentrations

Sample	% cell viability Concentration of sample			
	200 ug/ml	400 ug/ml	600 ug/ml	800 ug/ml
N. Control	100± 0.2	100± 0.3	100± 0.2	100± 0.4
PM	87± 0.5	74± 0.4	65± 0.7	42± 0.5
PH	91± 0.4	71± 0.5	67± 0.5	48± 0.6
GM	81± 0.5	75± 0.2	68± 0.3	50± 0.3
GH	84± 0.2	80± 0.3	70± 0.6	60± 0.5

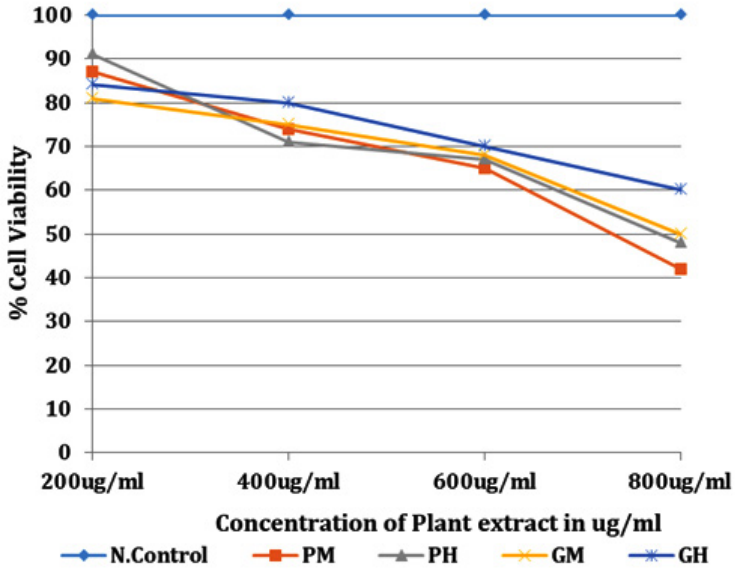

Fig. 5. Anticancer activity of different extracts against lung cancer cell line

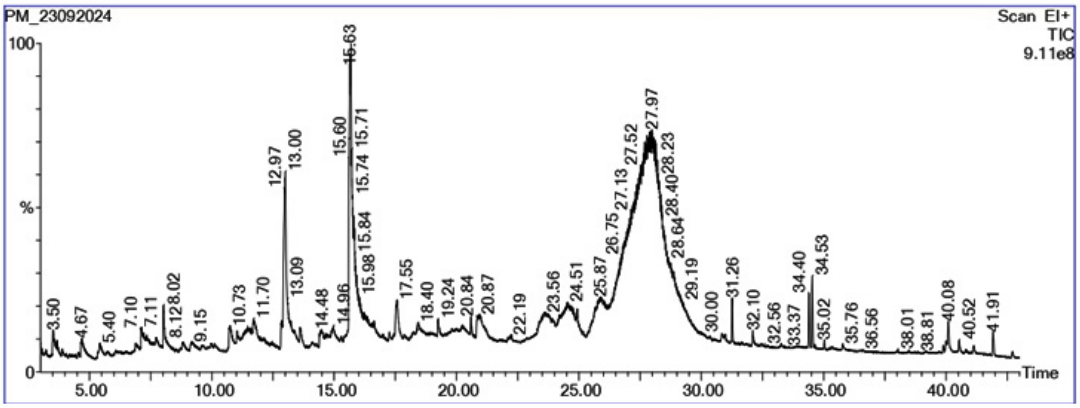

Fig. 6. The GC-MS chromatogram of the methanolic extract of *Withania coagulans* berries

berries (PH and PM) and *Psidium guajava* L. leaves (GH and GM). The antioxidant activity of these extracts at varying concentrations (10–50 mg/mL) is illustrated in Figure 2, showing a dose-dependent increase in activity across all groups.

Both water extracts (PH and GH) exhibit moderate and steady activity, with slightly higher values observed for *Psidium guajava* L. (GH) at higher concentrations. The results

Table 6. Identified compounds from *Withania coagulans* berry extract

Sr no.	RT	Compound name	Peak area %	Molecular weight g/mol
1	8.020	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.755	144.12532
2	13.003	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	3.754	144.13
3	15.684	5-Hydroxymethylfurfural	12.97	126.111
4	16.222	Imidazole-5-propionic acid, á-hydroxy-	0.74	156.14
5	17.561	1,3-Cyclohexanedione, 2-(2-propenyl)-	0.99	112.13
6	20.938	α-D-Mannofuranoside,1-O-(10-undecenyl)-	1.44	332.4
7	23.590	L-Mannose	1.23	180.156
8	24.545	Galacto-heptulose	1.85	210.18
9	25.871	Galacto-heptulose	4.13	210.18
10	27.973	Methyl-α-D-thiogalactoside	72.11	210.25

Table 7. Identified compounds from *Psidium guajava* L. leaf extract

Sr. no	RT	Compound name	Peak area %	Molecular weight(g/mol)
1	3.020	1,2-Propandiol difromate	1.22	132.11
2	9.266	Cis-Aconitic anhydride	2.25	156.09
3	12.935	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	4.542	144.12
4	15.604	5-Hydroxymethylfurfural	16.215	126.111
5	25.540	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-,	1.396	222.28
6	26.733	1,2,3,5-cyclohexentetrol	14.112	148.16
7	27.571	erythro-(cis)(1,4),(cis)(1',4')-4,4'- Dihydroxybicyclooctyl	21.12	252.38
8	28.488	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7atetrahydrobenzofuran-2(4H)-one	14.311	166.26
9	29.239	6-epi-shyobunol	7.47	222.36634
10	30.702	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9- diol	1.30	238.3657

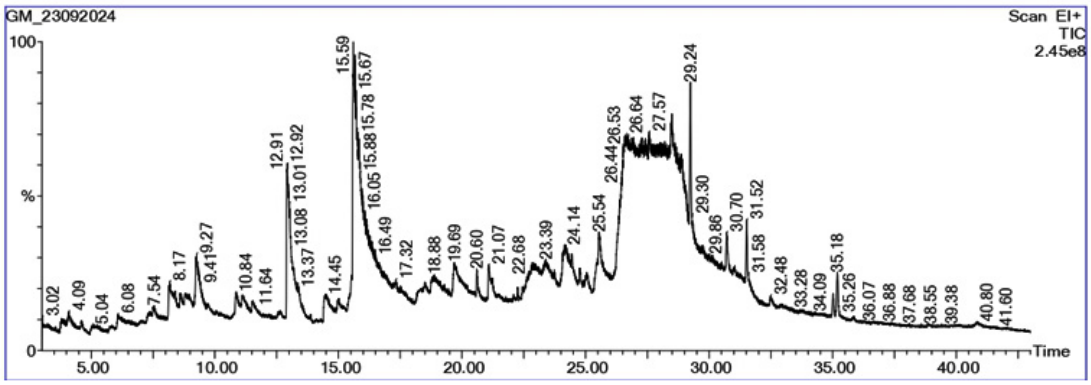

Fig. 7. The GC-MS chromatogram of the methanolic extract of *Psidium guajava* L. leaves

indicate that the plant extracts exhibit significant antioxidant potential, which increases with higher concentrations, as shown by the data derived from the graph and displayed in Table 2.

The methanol extract of *Psidium guajava* L.(GM) consistently exhibits the highest activity, reaching up to 95 ± 0.3 at 40 and 50 mg/mL. Similarly, the methanol extract of *Withania coagulans* (PM) shows a significant increase in activity with concentration, from 11 ± 0.5 at 10 mg/mL to 69 ± 0.4 at 50 mg/mL.

Anti-microbial Activity against *Staphylococcus aureus*

The antimicrobial test results, based on the zone of inhibition's diameter (measured in mm), indicate the effectiveness of the tested extracts against microbial growth. Larger inhibition zones correspond to higher antimicrobial activity, with the plant extracts demonstrating varying efficacy depending on the microbial strain and concentration. The results confirm that the plant extracts possess significant antimicrobial properties, supporting their potential use in combating microbial infections.

Table 4 and figure 3&4 presents the antimicrobial activity of water and methanol extracts of *Withania coagulans* berries (PH and PM) and *Psidium guajava* L. leaves (GH and GM) against *Staphylococcus aureus*, expressed as the diameter of the zone of inhibition which is in millimeters. The results indicate an increase in antimicrobial activity for all extracts which is concentration-dependent. Amongst the samples, the highest activity was exhibited by the methanol extract of *Withania coagulans* (PM), with a zone of inhibition reaching 35 ± 0.6 mm at 50 mg/mL. Similarly, the Psidium guajava L. (GM) methanol extract showed significant activity, achieving a zone of inhibition of 21 ± 0.3 mm at the same concentration.

In contrast, the water extracts (PH and GH) demonstrated lower antimicrobial activity, with PH reaching a maximum zone of inhibition of 16 ± 0.5 mm and GH 12 ± 0.2 mm at 50 mg/mL. These findings suggest that methanol extracts are more effective against *S. aureus* than their water counterparts, with PM showing the strongest antimicrobial potential among the tested extracts.

Anti-cancer Activity

Table 5 presents the cytotoxic effect of different plant extracts at varying concentrations, showing the percentage of cell viability for each extract (PM, PH, GM, and GH) against the A549 lung cancer cell line, as measured by the MTT assay. The data reveals a concentration-dependent cell viability decrease for all extracts, compared to the negative control (N. Control), which maintained 100% viability across all concentrations.

At concentrations of 200 µg/mL to 800 µg/mL, PM (methanol extract of Withania coagulans) and GM (methanol extract of *Psidium guajava* L.) demonstrated stronger cytotoxic effects, with cell viability reaching 87± 0.5% and 81± 0.5%, respectively, at 200 µg/mL and progressively decreasing at higher concentrations. In contrast, PH (water extract of *Withania coagulans*) and GH (water extract of *Psidium guajava* L.) showed slightly less potent effects, with cell viability ranging from 91± 0.4% at 200 µg/mL to 60± 0.5% at 800 µg/mL for GH.

At the highest concentration of 800 µg/mL, all extracts exhibited significant cytotoxicity, indicating their potential as candidates for therapeutic applications, particularly in anticancer treatments.(Figure 5) These results suggest that methanol extracts, especially PM and GM, may be more effective in inhibiting cell viability, warranting further investigation in cancer treatment research.

GC–MS

The GC-MS analysis of the methanolic extracts from *Psidium guajava* L. leaves and *Withania coagulans* berries identified a variety of bioactive compounds, including secondary metabolites with potential therapeutic properties. The results provide a strong basis for further pharmacological and biochemical studies to validate and utilize these bioactive compounds in the development of plant-based medicines.

Figure 6 provides the chromatogram of the methanolic extract of *Withania coagulans* berries, showcasing the retention times and peak area corresponding to the identified compounds. Similarly, Figure 7 depicts the chromatogram of the methanolic extract of *Psidium guajava* L. leaves, offering a clear graphical representation of the chemical profile.

The top 10 identified compounds from *Withania coagulans* berry extract are listed in Table 6, highlighting significant phytoconstituents.

Meanwhile, Table 7 enumerates the top 10 identified compounds from *Psidium guajava* L. leaf extract, emphasizing their relevance in Phytotherapeutic research. These findings emphasize the potential of both extracts as sources of bioactive compounds for biomedicine, paving the way for further research into their pharmacological properties and applications.

DISCUSSION

The study highlights the therapeutic potential of *Withania coagulans* berries and *Psidium guajava* L. leaves, with methanol extracts showing superior bioactivity across multiple assays. The methanol extract of *Psidium guajava* (GM) demonstrated the highest antioxidant activity due to its rich flavonoid content. Both GM and PM showed strong α-amylase inhibition, indicating potential anti-diabetic effects. Antimicrobial tests revealed that methanol extracts were more effective than water extracts, with PM exhibiting the strongest activity against *Staphylococcus aureus*. Similarly, anticancer assays against the A549 lung cancer cell line showed that PM and GM significantly reduced cell viability, highlighting their potential for further anticancer research.

The study by Aisha Ashraf et al. demonstrated that the methanol extract of *Psidium guajava* exhibited the highest antioxidant activity with an IC50 value of 89.82 ± 0.55 µg/Ml[16],followed by chloroform and hexane extracts with significantly lower activity. Our findings not only confirm the superior antioxidant potential of methanol extracts but also show even stronger activity at higher concentrations. The methanol extract of *Psidium guajava* (GM) in our study exhibited the highest DPPH scavenging activity, reaching 95 ± 0.3 at 40 and 50 mg/mL, outperforming previously reported values. Notably, the water extract of *Psidium guajava* (GH) displayed significant activity, increasing from 43 ± 0.8 at 10 mg/mL to 69 ± 0.7 at 50 mg/mL. Similarly, the water extract of *Withania coagulans* (PH) exhibited moderate but consistent activity, reaching 63 ± 0.2 at 50 mg/mL. While methanol extracts showed the highest potency, our study highlights that water extracts also possess substantial antioxidant properties, making them a valuable and eco-friendly alternative for potential therapeutic applications.

The study by Mayur Ram et al. highlighted the antidiabetic and antioxidant potential of *Withania coagulans*[17]. Similarly, our study demonstrated strong antioxidant activity, with methanol extract (PM) increasing from 11 ± 0.5 at 10 mg/mL to 69 ± 0.4 at 50 mg/mL, while the water extract (PH) reached 63 ± 0.2 at 50 mg/mL. The methanol extract (PM) showed a higher antimicrobial effect against *Staphylococcus aureus* (35 ± 0.6 mm) than the water extract (PH) (16 ± 0.5 mm). Cytotoxicity assays confirmed significant reduction in cell viability, with PH and PM exhibiting dose-dependent anticancer effects. PH showed 91 ± 0.4% cell viability at 200 µg/mL, while PM reached 87 ± 0.5%. These findings highlight the strong pharmaceutical potential of *Withania coagulans*, with both methanol and water extracts demonstrating promising antioxidant, antimicrobial, and cytotoxic activities.

The GC-MS analysis provided a detailed chemical profile of the methanolic extracts, identifying bioactive compounds with potential therapeutic properties. For example, the methanol extract of *Withania coagulans* contained 5-Hydroxymethylfurfural and Methyl-á-D-thiogalactoside,[18] which are known for their antioxidant and antimicrobial activities. Similarly, the methanol extract of *Psidium guajava* contained 6-epi-shyobunol and other significant secondary metabolites, which may contribute to its pharmacological effects.[19] These findings suggest that these extracts are valuable sources of bioactive compounds for developing plant-based medicines.

CONCLUSION

This study underscores the significant therapeutic potential of *Withania coagulans* berries and *Psidium guajava* L. leaves, particularly their methanol extracts, in addressing oxidative stress, microbial infections, diabetes, and cancer. The high antioxidant activity, strong á-amylase inhibition, and potent antimicrobial and anticancer effects highlight their suitability for phytotherapeutic applications. GC-MS analysis identified bioactive compounds that may underlie these activities, paving the way for further pharmacological and biochemical investigations. These findings reinforce the role of plant-based extracts as promising candidates for developing natural, multi-targeted therapeutic agents.

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The authors do not have any conflict of interest.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials**.**

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Not Applicable**.**

Authors' Contribution

Kruti Dave conceptualized and designed the study, conducted the experimental work, and contributed to data analysis and manuscript preparation; Birva Limbachiya: assisted with the phytochemical screening, antidiabetic, and antioxidant activity assays, and provided critical input for data interpretation; Heer Tank: conducted antimicrobial and anticancer activity assays and contributed to the GC-MS profiling and result analysis. All authors reviewed and approved the final manuscript.

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