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RESEARCH ARTICLE

Assessment of Synergistic Antioxidant Activity of Psidium guajava and Cynodon dactylon Extracts with Molecular Docking Analysis for Antidiabetic Potential

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Abstract: In this research, the synergistic antioxidant activity and antidiabetic activity in Cynodon dactylon (Bermuda grass) and Psidium guajava (guava) are evaluated by combining phytochemical study, antioxidant assays, and in-silico molecular docking studies. The plants, conventionally utilized worldwide, were scientifically assessed for pharmacognostical, physicochemical, and phytochemical profiling. Ethanolic extracts of both plants were obtained and blended in similar amounts for combined examination. Initial phytochemical screening established the presence of active compounds like flavonoids, tannins, phenolic compounds, alkaloids, and cardiac glycosides—most of which are renowned, for their potent antioxidant and therapeutic activities. The DPPH radical scavenging activity was tested where the combined extract had an extensive inhibition value of up to 89.22%, depicting enormous free radical scavenging potential even in minute concentrations. Chemical fingerprinting of the extracts was established via Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) revealing the occurrence of several classes of phytochemicals under UV detection at wavelengths 254 nm and 366 nm. Molecular docking was performed using bioactive compounds quercetin, kaempferol, and luteolin against the AMPK protein (PDB ID: 2H6D), an extensively reported cellular energy homeostasis regulator and antidiabetic target. The maximum binding of kaempferol with -8.29 kcal/mol followed by quercetin and luteolin validating the antidiabetic activity of the extract confirms its potential therapeutic significance. Physicochemical parameters like loss on drying, ash value, and solubility analysis also validated the purity and quality of the plant materials. The synergy of in-vitro and in-silico methods validates the efficacy of the combined extract for future pharmacological application. The study concludes that the synergistic interaction of Psidium guajava and Cynodon dactylon offers a promising natural choice for antioxidant and antidiabetic therapy, which warrants further study through compound isolation and clinical validation.

Keyword: Phytochemical screening, DPPH assay, Synergistic extract, Antidiabetic potential, Antioxidant activity.

INTRODUCTION

In the search for efficient and safer alternatives to synthetic medicines medicinal plants have once again received attention from science for their wealth of phytochemicals and age-old use as therapeutic agents[1][2]. Among them Psidium guajava (guava) and Cynodon dactylon (Bermuda grass) are interesting candidates that exhibit strong antioxidant and antidiabetic activities[3][4]. Psidium guajava, a plant of the Myrtaceae family, is cultivated extensively in tropical and subtropical countries and is reported to have traditional uses in the treatment of cough, diarrhea, hypertension, and diabetes mellitus[5][6]. Guava leaves, especially, contain high amounts of bioactive compounds like quercetin, kaempferol, tannins, and vitamin C, which possess strong free radical scavenging and antidiabetic activities[7][8]. Recent research articles in Research Journal of Pharmacy and Technology (RJPT) have confirmed the plant's pharmacological effects, which include its ability to prevent oxidative stress as well as increase glucose uptake through the modulation of insulin signaling pathways[9][10]. In the same vein, Cynodon dactyon, a Poaceae family perennial grass, has been hallowed traditionally in Ayurvedic medicine for its therapeutic uses such as antimicrobial, anti-inflammatory, and antioxidant activities[11][12][60].

Phytochemical studies confirm the occurrence of flavonoids such as luteolin and kaempferol, saponins, and alkaloids, which are responsible for its medicinal value[13][14]. Recent RJPT reports and supported studies indicate that the grass has high free radical scavenging activity and antidiabetic activity due to its insulin-mimetic action and antioxidant enzyme induction[15][16]. Synergy of these two plants is of specific scientific significance because their combination will likely enhance therapy by complementary phytoconstituents[17][18]. Even though individual

values are well established, not many studies have looked into the synergistic activity of P. guajava and C. dactylon together, especially with existing methodologies such as molecular docking and HPTLC analysis[19][20]. This work seeks to close that gap by assessing the antioxidant activity of the combined extracts and their antidiabetic potential via in-vitro assays and in-silico modeling against AMPK—a regulatory enzyme of energy homeostasis and an established target for antidiabetic therapy[21][22]. By combining traditional knowledge with modern pharmacological tools, this work enhances the scientific validation and potential utilization of natural phytotherapeutics[23][24][59].

Table 1: Botanical classification of Psidium guajava[5][6]

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta Vascular plants
Sub division	Spermatophyta Seed plants
Division	Magnoliophyta Flower plants
Class	Magnoliopsida Dicotyledonous
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Gender	Psidium
Species	Psidium guajava

Table 2: Botanical classification of Cynodon dactylon[11][12]

Kingdom	Plantae
Sub Kingdom	Tracheobionta
Sub Division	Spermatophyta
Division	Magneliophyta
Class	Liliosidae
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae
Genus	Cynodon
Species	Cynodon dactylon

Psidium guajava

In article 2010 by A. M. Metwally et al[7] proposed that leaf extract of guava is used in many pharmaceutical preparations as a cough sedative, analgesic, anti-inflammatory properties, antimicrobial, hepatoprotective, Guava leaf extract contains flavonoids, mainly quercetin derivatives, which are hydrolyzed in the body to give the aglycone quercetin which is responsible for the spasmolytic activity of the leaves.[7][8] This Quercetin has several pharmacologic actions; it inhibits the intestinal movement, reduces capillary permeability in the abdominal cavity and has many property such as anti-tumor, anti-inflammatory, anti-viral.[8][9] Guava also has antioxidant properties which are attributed to the polyphenols found in the leaves.[6][9] Guava main chemical include alanine, ascorbic acid, ascorbigen, benzaldehyde, butanal, carotenoids, caryophyllene, catechol7tannins, crataegolic acid, D7galactose, D7galacturonic acid, ellagic acid, ethyl octanoate, essential oils, flavonoids, gallic acid, glutamic acid, goreishic acid, guafine, guavacoumaric acid, guaijavarin, guajiverine, guajivolic acid.[5][8][10]

Table 3: Various nutrient composition present in Psidium guajava[5][8]

Nutrient	Composition
Protien	0.1-0.6 mg
Mositure	2.79- 5.5 g
Lipid	0.43-0.7 mg
Fibre	0.9-1 g
Carbohydrate	9-17 mg
Iron	200-400.I.U
Calcium	17.9-30 mg
Phosphorus	0.3-0.7 mg
Thiamine	0.03-0.05 mg
Riboflavin	0.6-1 mg
vitamin C	228 mg

vitamin A	0.047 mg

Cynodon dactylon

While in a recent year a number of studies have been carried out which proved that there were many phytochemical components in Bermudagrass influencing its healing properties.[12][13][14] These phytochemicals include alkaloids, saponins, and flavonoids.[12][15] Flavonoids such as quercetin and kaempferol are known to have anti-inflammatory properties by reducing inflammation and dampening the process of oxidative damage to cells, displaying powerful anti-inflammatory and antioxidant properties.[14][16] Cynodine, an alkaloid obtained from Bermudagrass, was reported to show analgesic and anti-tumor activities, thus suggesting its medicinal potential.[12][17] Bermudagrass has widely been utilized in the management of gastrointestinal-related diseases, including diarrhea and gastrointestinal infections, due to its strong antibacterial and anti-inflammatory activity.[11][12][17][62] Contrarily, research is being conducted to explore its possible use in the management of chronic diseases, including diabetes and cardiovascular disease, due to the bioactive compounds in its antioxidant and anti-inflammatory activities.[12][16][18]

MATERIAL AND METHODS

1. Plant Sample Collection

Fresh guava leaves were collected from healthy guava tree and fresh Bermuda grass were collected from Grass land, the samples were visually observed for any kind of infection, spores, damage, discoloration, and distortion first and then shade dried for 7 days.[19][20]

Fig.1 Dried Guava Leaves

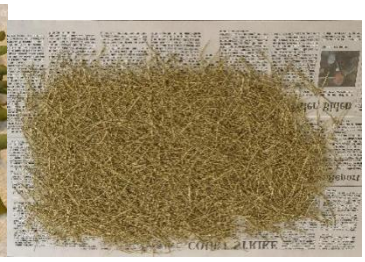


Fig.2 Dried Bermuda Grass

After 7 days the midribs of leaves were removed, and both the samples were grinded and passed on sieve no.44 to get a fine powder.[19]

Fig.3 Fine Powder of Bermuda Grass

Fig.4 Fine Powder of Guava Leaf

2. Preparation of plant extract

The herbal powders were mixed (50:50 ratio) in ethanol with sample-ethanol ratio of 1:3 for phytochemical analysis and kept for 48hrs in dark. Occasion stirring is done.[21]
After 48 hrs the extract is filtered on Whatman filter paper to enhance the purity of the filtration, marked and the obtained filtrate is stored in the refrigerator. [21][22]

Fig. 5 Ethanolic Extract

In other volumetric flask 10g powder and 100ml methanol are combined to obtain extract for antioxidant activity DPPH assay and centrifuged for 2hr 30 mins, supernatant solution is filtered, labelled and stored in refrigerator. [9][23][24]

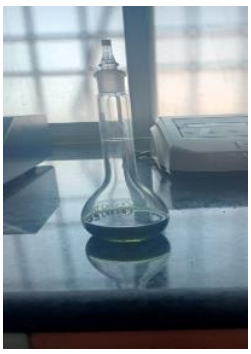


Fig.6 Methanolic Extract

3. DPPH Assay

DPPH Stock Solution: 4mg DPPH in 100ml standard volumetric flask, make up the volume with methanol.[25][26]
From 10 mole methanolic extract, 1ml is taken and make up to 10ml standard flask to get 10ml of 1mol solution, from the 1mol solution 1ml is taken and make up to 10ml in standard flask to get 10ml of 0.1mol,[9][25][26] shown in Fig.7 .

Fig.7: Test tube containing sample and DPPH

- This sample is analysed in UV-Vis Spectroscopy at 517nm to find the absorbance of the sample[26][27]
- The %inhibition of antioxidant is compared with standard ascorbic acid solution.[28][29][63]
- 4. Phytochemical analysis
- Phytochemicals such as Alkaloids, Cardiac Glycoside, Phenolic compounds, Tannins, Flavonoids, Carbohydrates, Anthraquinone Glycoside, Protein, Steroids, are analysed by various Phytochemical Identification test.[30][31][32][33]
- Determination of Alkaloids
- Dragendorff test: Take 0.5ml ethanolic extract in a test tube and add 0.5ml dil. HCl and Dragendorff reagent.[30]
- Determination of Cardiac Glycoside
- Killer Killani test: Take 0.5 ml ethanolic extract and few drops of ferric chloride and conc. H2SO4 in a test tube.[31]
- Baljet test: Take 0.5ml ethanolic extract and 0.2g of sodium picrate in test tube.[31]
- Determination of Phenolic compounds and Tannins
- Lead acetate test: Take 0.5 ml ethanolic extract and 10% lead acetate solution in a test tube.[30]

- Determination of Flavonoid
- Lead acetate test: Take 0.5 ml ethanolic extract and 10% lead acetate solution in a test tube[31]
- Shinoda test: Take 0.5ml ethanolic extract and 3ml 95% ethanol, few drops of Con. HCl and Few magnesium turnings in a test tube[34]
- Determination of Carbohydrates
- Fehlings test: Take 0.5ml ethanolic extract in test tube and add 0.5ml Fehling A and 0.5ml of Fehling B and heat[30]
- Determination of Anthraquinone Glycoside
- Brontrager test: Take 0.5ml ethanolic extract in test tube and in it add few drops of dil HCl and boil then add 0.5ml of chloroform or benzene and shake well, add few drops of ammonia[30][64]
- Determination of Protein
- Biuret Test: Take 0.5ml ethanolic extract in test tube and to it add 40% NaOH and few drops of CuSO4 solution[30]
- Ninhydrin test: Take 0.5ml ethanolic extract in test tube and to it add Ninhydrin Reagent[30]
- Determination of Steroids
- Salkowski test: Take 0.5ml ethanolic extract in test tube and to it add 0.5ml Chloroform, 0.5ml conc. H2SO4 and shake[30][31]

5. TLC Test

Take 5g of silica gel in a beaker and make it into slurry by distilled water. Apply the slurry on two glass slide by pour method and dired.[35][36] Mobile solvent is prepared which has n-butanol, distilled water, acetic acid in ratio 4:5:1, and left for saturation for 30 mins.[35][36]

The samples were applied on the plate via capillary tube. The Plates were kept in solvent and left till the samples travel 3/4th of plate. The plates are dried and ferric chloride reagent on one TLC plate, and conc. Nitric acid on other TLC plate.[35][61]

6. In-Silico Analysis

Five chemical constituents that exhibits antioxidant activity present in guava and Bermuda grass were taken each, and a common protein AMPK was chosen.[37][38][39]

Chemical constituents were taken from PubChem, and protein was taken from PDB website.[40][37][39]
The protein that is chosen has following characteristic: Found in Homo-Sapiens, Non-Mutated, Refinement Resolution (Å) is between 1.5-2.0.[39]

Docking is done via Auto Dock.[41][42][65]

Molecule are viewed through Molegro Molecular Viewer.[43]

7. Loss on Drying

Weight of empty petri dish was recorded. 0.5g of Guava powder and 0.5g Bermuda grass powder were taken in the petri dish and weighed. The petri dish is then kept in hot air oven for 1 hr, after an hour the petri dish is weighed and its before and after weigh is recorded.[44][45]

8. Ash Value

Empty weight of crucible was recorded. 0.5g of guava powder and 0.5g Bermuda grass powder were taken in the crucible and weighed. The crucible is kept in muffle furnace for 24hr. After 24hr the crucible is weighed and the ash value is recorded.[46][47]

9. Acid Insoluble and Water Soluble test

0.4g of ash is taken and dissolved in 2N conc. HCL (14ml of hcl made upto 25ml water) and 25ml water respectively, the solution is filtered and the filter paper is weighed and again kept in muffle furnace.[46]

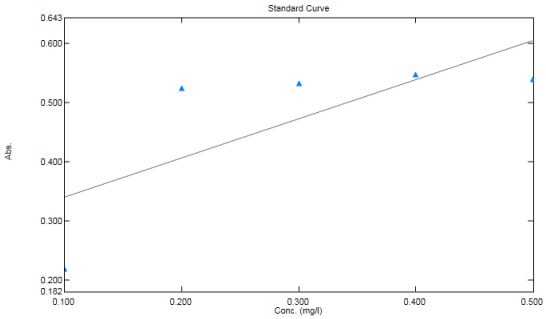
10. Plant Authentication and other test

- Authentication certificate was received from Siddha Central Research Institute (SCRI) Department of Pharmacognosy, Aruambakkam, Chennai, Tamil Nadu.[48]
- Authentication No. 1125.28012510, 1125.28012509
- Samples were given for the following tests: Morphology, Powder microscopy, quantitative, Microscopy.[49]

11. HPTLC Analysis

- HPTLC analysis was performed for Ethanolic Extract Sample[35][36][50]
- Results
- DPPH Assay
- Table 4: Results of UV Spectroscopy
- Control absorbance= 2.041

No.	Sample ID	Concentration (mg/ml)	Abs (517nm)	Percentage of inhibition
1	Sample 1	0.1	0.22	89.22%
2	Sample 2	0.2	0.525	74.27%
3	Sample 3	0.3	0.533	73.88%
4	Sample 4	0.4	0.539	73.59%
5	Sample 5	0.5	0.547	73.19%



Phytochemical Analysis

Table 5: Results of Phytochemical Analysis

Test	Observation	Conclusion
Test for Alkaloids		
Dragondroff test: 0.5ml extract + 0.5ml dil. HCl + Dragondroff reagent	Orange brown precipitate	+
Cardiac Glycoside test:		
Killer Killani test: 0.5 ml extract + few drops of ferric chloride and conc. H2SO4	Reddish brown colour	+
Baljet test: 0.5ml extract + 0.2g of sodium picrate	Brownish-Yellow colour	+
Test for phenolic compounds, tannins		
Lead acetate test: 0.5 ml extract + 10% lead acetate solution	White precipitate	+
Test for Flavonoids		
Lead acetate test: 0.5 ml extract + 10% lead acetate solution	Yellow Precipitate	+
Shinoda test: 0.5ml extract + 3ml 95% ethanol + few drops of Con. HCl + Few magnesium turnings	Brownish-Pink colour	+
Test for Carbohydrates		
Fehlings test: 0.5ml extract + 0.5ml Fehling A + 0.5ml of Fehling B and heat	Brick red precipitate	-
Antraquinone Glycoside test		
Brontrager test: 0.5ml extract + few drops of dil HCl and boil and add 0.5ml of chloroform or benzene and shake well, add few drops of ammonia	Ammonical layer turns pink	-
Test for Protein		
Biuret Test:	Produces blue colour	-

0.5ml extract + 40% NaOH and few drops of CuSO4 solution		
Ninhydrin test: 0.5ml extract + Ninhydrin Reagent	Purple or bluish colour	-
Test for Steroids		
Salkowski test: 0.5ml extract + 0.5ml Chloroform + 0.5ml conc. H2SO4 and shake	Chloroform layer turns red	-

Observation

From above result it indicates that guava-bermuda grass ethanolic extract contain the following phytochemical Flavonoids, Phenolic compounds, Tannins, Alkaloids, and Cardiac Glycoside, which has good antioxidant activity, except cardiac glycoside where some studies says it may indirectly shows antioxidant activity.[30][31][32][33][54]

TLC

Fig.8 TLC with Conc.HNO3 Reagent **Fig.9 TLC Plate with FeCl3 Reagent**

Table 6: Results of TLC analysis

Ferric Chloride Reagent			
Sample	Distance of Solvent Phase	Distance of sample	RF
Grass Extract	4.5ml	3.5ml	0.77
Guava Extract		3ml	0.66
Mixture Extract		3.5ml	0.77
Conc. Nitric Acid			
Grass Extract	4.5ml	3.2ml	0.71
Guava Extract		2.7ml	0.6
Mixture Extract		3.3ml	0.73

- **Observation**
- Higher Rf (e.g., 0.77) suggests the compound is less polar (it travels farther with the solvent).
- Lower Rf (e.g., 0.60) means the compound is more polar (sticks to the stationary phase more).
- Inference:
- Guava extract generally shows lower Rf than grass, meaning more polar compounds might be present in guava (e.g., tannins or phenolics).
- The mixture extract's Rf values are close to the grass extract—suggesting grass might dominate the mobility pattern in the mixture.[35][36][58]
- In-Silico Analysis

Table 7: Protein that is taken from PDB website

S.No	Name	Structure	Dock Structure

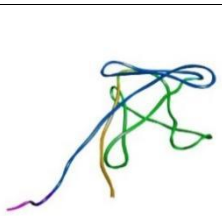
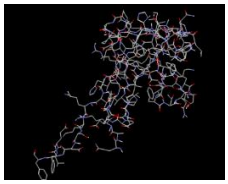
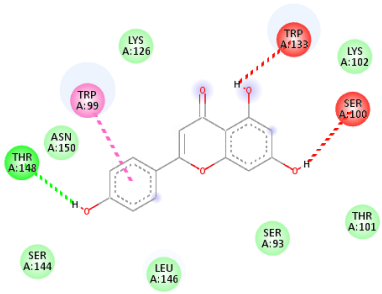
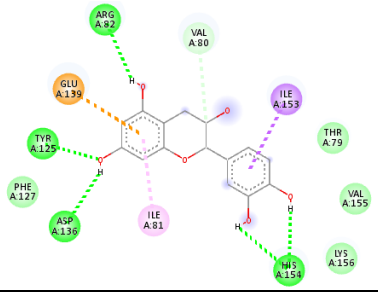
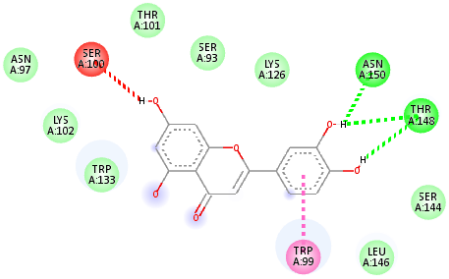
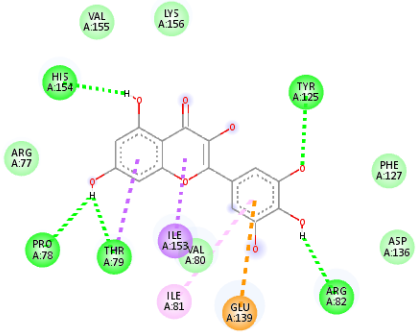
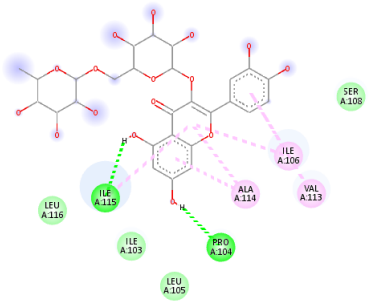
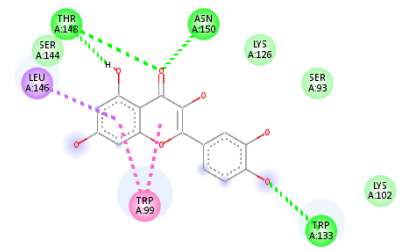
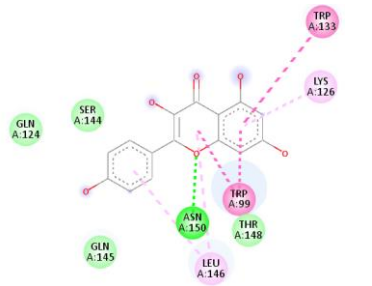
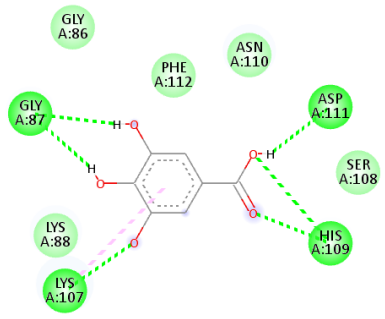
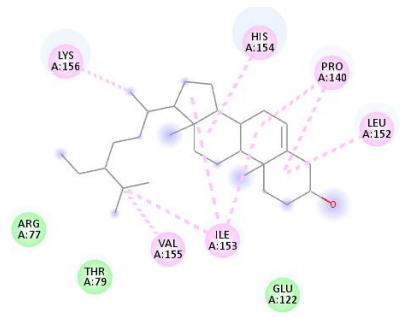
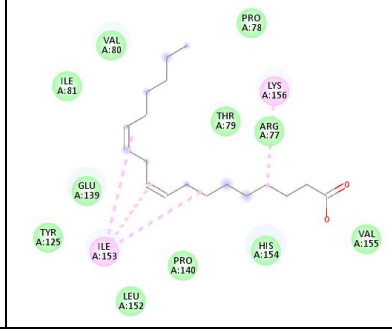
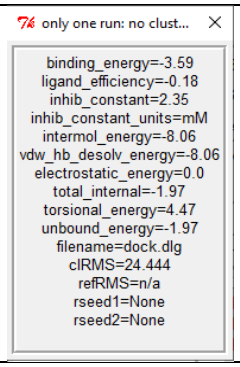
1	**AMPK (pdb_00002h6d)**		

Table 8: Binding energy and binding sites of ligands used

Ligand + Protein	Binding Site	Binding Energy
AMPK+ Apigenin		only one run: no clust... binding_energy=-7.35 ligand_efficiency=-0.37 inhib_constant=4.06 inhib_constant_units=uM intermol_energy=-8.55 vdw_hb_desolv_energy=-8.55 electrostatic_energy=0.0 total_internal=-1.03 torsional_energy=1.19 unbound_energy=-1.03 filename=dock.dlg clRMS=27.102 refRMS=n/a rseed1=None rseed2=None
AMPK+ Catechin		only one run: no clus... binding_energy=-7.01 ligand_efficiency=-0.33 inhib_constant=7.31 inhib_constant_units=uM intermol_energy=-8.8 vdw_hb_desolv_energy=-8.8 electrostatic_energy=0.0 total_internal=-1.55 torsional_energy=1.79 unbound_energy=-1.55 filename=dock.dlg clRMS=24.729 refRMS=n/a rseed1=None rseed2=None
AMPK+ luteolin		only one run: no clust... binding_energy=-7.87 ligand_efficiency=-0.37 inhib_constant=1.7 inhib_constant_units=uM intermol_energy=-9.36 vdw_hb_desolv_energy=-9.36 electrostatic_energy=0.0 total_internal=-2.1 torsional_energy=1.49 unbound_energy=-2.1 filename=dock.dlg clRMS=26.942 refRMS=n/a rseed1=None rseed2=None
AMPK+ Myricetin		only one run: no clust... binding_energy=-7.54 ligand_efficiency=-0.33 inhib_constant=2.98 inhib_constant_units=uM intermol_energy=-9.63 vdw_hb_desolv_energy=-9.63 electrostatic_energy=0.0 total_internal=-3.25 torsional_energy=2.09 unbound_energy=-3.25 filename=dock.dlg clRMS=24.266 refRMS=n/a rseed1=None rseed2=None

AMPK+ Rutin		only one run: no clust... binding_energy=-3.2 ligand_efficiency=-0.07 inhib_constant=4.48 inhib_constant_units=mM intermol_energy=-7.98 vdw_hb_desolv_energy=-7.98 electrostatic_energy=0.0 total_internal=-13.02 torsional_energy=4.77 unbound_energy=-13.02 filename=dock.dlg cIRMS=14.958 refRMS=n/a rseed1=None rseed2=None
AMPK+ Quercetin		only one run: no clust... binding_energy=-7.94 ligand_efficiency=-0.36 inhib_constant=1.52 inhib_constant_units=uM intermol_energy=-9.73 vdw_hb_desolv_energy=-9.73 electrostatic_energy=0.0 total_internal=-2.71 torsional_energy=1.79 unbound_energy=-2.71 filename=dock.dlg cIRMS=27.362 refRMS=n/a rseed1=None rseed2=None
AMPK+ Kaempferol		only one run: no clust... binding_energy=-8.29 ligand_efficiency=-0.39 inhib_constant=843.43 inhib_constant_units=nM intermol_energy=-9.78 vdw_hb_desolv_energy=-9.78 electrostatic_energy=0.0 total_internal=-1.64 torsional_energy=1.49 unbound_energy=-1.64 filename=dock.dlg cIRMS=28.899 refRMS=n/a rseed1=None rseed2=None
AMPK+ Gallic acid		Conformation 1 Info binding_energy=-6.59 ligand_efficiency=-0.55 inhib_constant=14.81 inhib_constant_units=uM intermol_energy=-8.08 vdw_hb_desolv_energy=-8.08 electrostatic_energy=0.0 total_internal=-1.99 torsional_energy=1.49 unbound_energy=-1.99 filename=dock.dlg cIRMS=0.0 refRMS=26.07 rseed1=None rseed2=None
AMPK+ Beta-sitosterol		only one run: no clust... binding_energy=-7.4 ligand_efficiency=-0.25 inhib_constant=3.77 inhib_constant_units=uM intermol_energy=-9.49 vdw_hb_desolv_energy=-9.49 electrostatic_energy=0.0 total_internal=-1.05 torsional_energy=2.09 unbound_energy=-1.05 filename=dock.dlg cIRMS=28.096 refRMS=n/a rseed1=None rseed2=None

AMPK+ Linoleic acid		

Observation

Kaempferol showed the strongest binding with AMPK (binding energy: -8.29 kcal/mol), indicating it could be the most potent AMPK modulator among the compounds tested and has high antidiabetic action[37][41][42]

Luteolin (-7.87) and Quercetin (-7.94) also exhibited strong binding affinities, suggesting good potential as AMPK activators/inhibitors.[37][38][42][43]

Rutin (-3.2) had the weakest binding energy, suggesting it may be less effective in modulating AMPK activity in this model.[39][42]

Common Binding Residues:

- His154, Thr148, Tyr125, Arg82 are frequently involved in binding across different ligands.
- These residues might be key interaction points or active site components of the AMPK protein.
- Loss on Drying
- Weight of empty petri dish (a): 92.76g
- Weigh of sample taken (b): 1g
- Weight of both petri dish and sample (c): 93.76g
- Weight of both petri dish and sample after drying (d): 93.65g
- Loss on Drying= c-d*100/b
- Results= 11%
- Ash Value
- Weight of empty crucible (a): 37.85g
- Weigh of sample taken (b): 1g
- Weight of both crucible and sample (c): 38.85g
- Weight of both crucible and ash (d): 37.91g
- Ash Value= (d-a/b) * 100
- Results= 6%

Observation

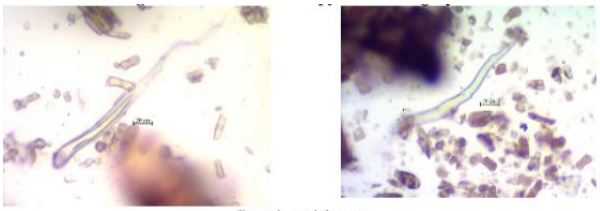
- The Sample has moderate mineral load, which is typical for many medicinal plant materials.[44][45][46][47]
- Acid insoluble and Water-soluble Value
- Acid Insoluble
- Weight of empty crucible: 37.85g
- Weight of crucible and residue: 39.13g
- Weight of crucible and residue ash: 37.86g
- Acid insoluble = (weight of residue/original weight) * 100
- Results= 0.78%
- Observation
- The sample has minimal contamination, indicating good handling and processing.[46][47]
- Water Soluble
- Weight of empty crucible (w1): 39g
- Weight of crucible and residue (w2): 40.25g
- Weight of crucible and residue ash (w3): 39.04g
- Water soluble = (W2-W1) – (W3-W1) * 100/ (W2-W1)
- Results= 96.8%

- Indicates a high percentage of minerals are water-soluble, which may include potassium, sodium, and magnesium salts.[46]
- Morphology, Powder microscopy, quantitative, Microscopy.

Morphology:

Guava

- Fresh leaves are light green, simple, oblong to elliptic, margin entire, apex obtuse to
- bluntly acuminate; base rounded to sub cuneate; measuring 8 to 15 cm long and 4 to 6
- cm wide; petiole short; with characteristic odour and taste
- Bermuda Grass
- Stem, tender and narrow, nodes swollen and solid, internodes hollow, glabrous and shiny, 1
- to 3 cm in length and 1 to 3 mm in diameter, yellowish green with characteristic odour and
- no taste,
- Leaf - simple, alternate, distichous and ribbon shaped, long, narrowly linear, 2 to 10 cm in length, 2 to 3 mm in width, entire lower leaves usually flat, upper complicate, parallel veined, with sheathing leaf bases attached at the node and surrounding the internode like a tube to varying lengths, ligule very short, hairy and erect with characteristic odour and no tasteless[48][49]
- Powder Microscopy
- Guava Leaf
- The powder is green coloured with characteristic odour and taste; shows the characters like unicellular covering trichomes, surface view of upper epidermis, surface view of lower epidermis with paracytic, sectional view of palisade cells, fibre bundle, cluster and prismatic crystals



Covering trichomes

Fig.10 Powder microscopy of Psidium guajava leaf

Bermuda Grass

The chaff green powder shows papillose epidermis, unicellular trichome, epidermis with dumb mesophyll cells, parenchyma, silica crystals, starch grains, tracheid, fibres, and reddish content, silica crystals, starch grain with an aromatic odour and no characteristic taste.[48][49]

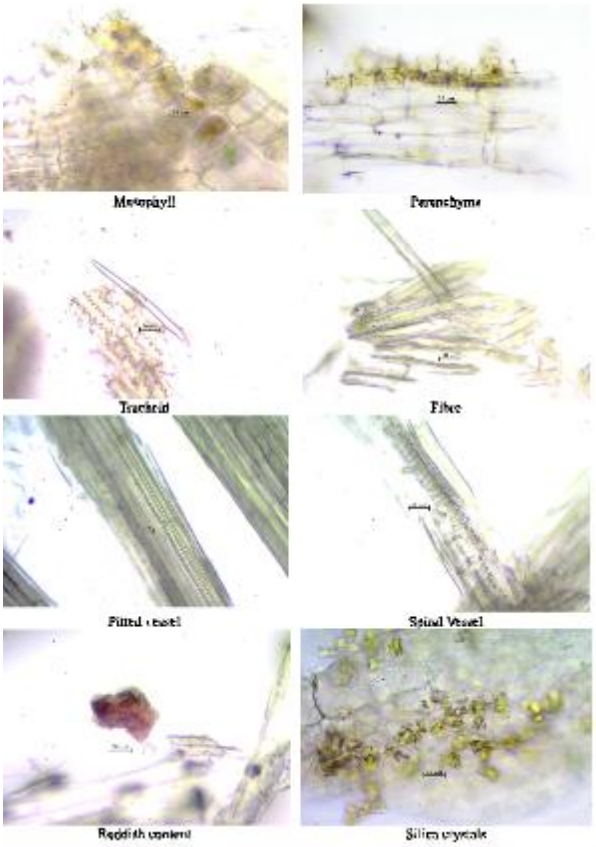
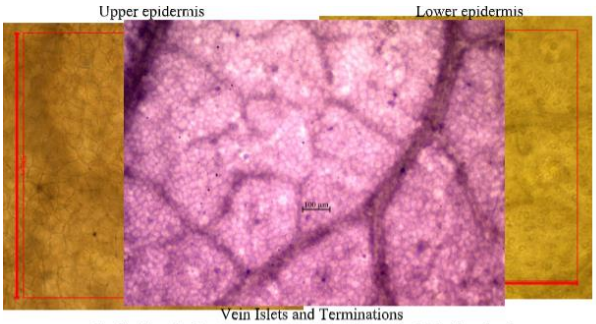


Fig.11 Powder microscopy of Cyanodon datylon aerial parts

Quantitative Microscopy of Guava

Table 11: Quantitative Microscopy of Psidium guajava[48]

Parameter	Upper Epidermis (/mm²)	Lower Epidermis (/mm²)
Epidermal Number	760 – 800	1300 – 1350
Stomatal Number	–	690 – 700
Stomatal Index	–	34.1 – 34.7
Palisade Ratio	7 – 8	–
Vein Islet Number	4 – 7	–
Vein Termination Number	2 – 4	–



E - Epidermis; **St** - Stomata; **VI** - Vein Islet; **VT** - Vein Termination

Fig.12 Quantitative microscopy of leaf of Psidium guajava

Microscopy

TS of leaf shows upper narrow concave and lower broad rounded wavy surfaces in the midrib with lateral ribbon like laminar extensions.

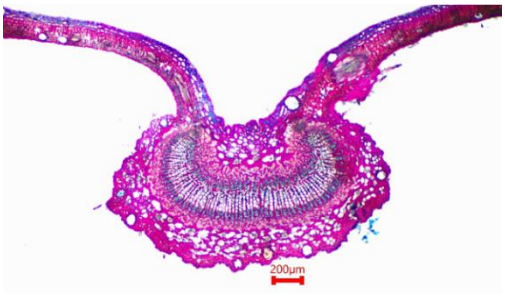


Fig.13 T.S of Psidium guajava leaf

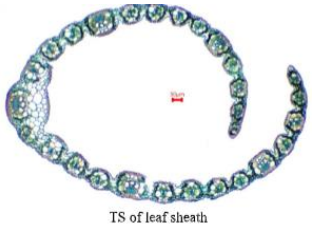


Fig.14 TS of Cyanodon dactylon leaf sheath and leaf

HPTLC

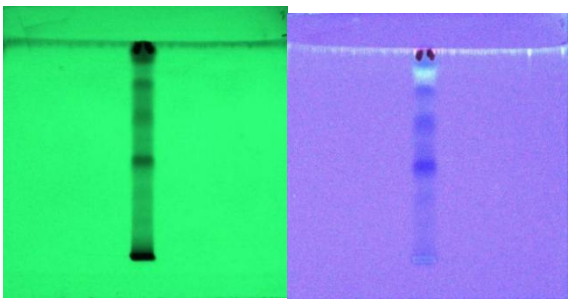


Fig.15: 254nm UV light (Shortwave) **Fig.16: 366nm UV light (Longwave)**

Observation:
In UV light 254nm it indicates presence of UV-active compounds, likely aromatic rings or conjugated double bond systems. In UV light 366nm it Shows presence of compounds with natural fluorescence (like flavonoids, coumarins, etc.).[50][55]

DISCUSSION

The synergistic antidiabetic and antioxidant effect of Psidium guajava and Cynodon dactylon was adequately assessed using an integrated approach with phytochemical profiling, DPPH assay, TLC, HPTLC, and molecular docking studies.[3][4][9][25][35][37] The DPPH assay (Table 4) substantiated that the mixed extract possessed notable free radical scavenging potential with a brilliant 89.22% inhibition[25][26][51] at the least concentration (0.1 mg/mL). The inhibition was higher than the usual single-plant extracts reported in the literature[9][13][56], supporting the likely synergistic enhancement when P. guajava and C. dactylon are mixed[17][18]. The lower absorbance value of Sample 1, as evident in Fig. 7, is confirmatory of the high antioxidant activity, indicating the plant extracts are effective even at very low doses. The phytochemical content (Table 5) supported the flavonoids, phenolic compounds, tannins, alkaloids, and cardiac glycosides in the ethanolic extract, all of which are well known to be contributors to antidiabetic and antioxidant activity[5][8][9][30][32][33]. This is consistent with the previous literature reporting flavonoid richness of P. guajava (quercetin, kaempferol) and alkaloid and flavonoid profile of C. dactylon Inadequacy of proteins and steroids, however, means that biological activities are likely to be driven by secondary metabolites rather than nutritional factors. The TLC profiling (Table 6; Figs. 8 and 9) revealed varied Rf values among various extracts and detecting reagents, with the mixture extract reflective of patterns close to C. dactylon. This observation reveals that C. dactylon components are likely to be more polar under the used solvent system, which could influence the extract profile of available compounds for biological activities[35][36]. The HPTLC results (Figs. 15 and 16) established the UV-active and fluorescence compounds present and indicated the aromatic phytochemical richness of the plant in coumarins and flavonoids[35][50][55]. Molecular docking studies (Tables 7 and 8) gave mechanistic insights into plant extracts' antidiabetic action. Kaempferol had the greatest binding affinity with AMPK (-8.29 kcal/mol), followed by quercetin and

luteolin, suggesting that the flavonoids are effective modulators of energy metabolism. The detection of common interacting residues (His154, Thr148, Tyr125, Arg82) among different ligands reflects the robustness of the docking model and points towards the identification of specific sites to be targeted for therapeutic interventions. Interestingly, high binding energies so observed are comparable to or better than those reported in earlier molecular docking studies using natural AMPK activators[37][41][42][43]. The physicochemical analyses, including loss on drying (11%) and ash value (6%), revealed good moisture content and mineral load, reflecting quality and stability of plant material used (Ash and Loss on Drying sections). Also,the low acid insoluble ash (0.78%) and high water-soluble mineral content (96.8%) reflect good processing practices and suggest high bioavailability of key minerals[44][46][47]. Microscopy examination (Figs. 10–14; Table 11) validated the morphological genuineness of the raw plant materials, thus establishing the credibility of the study results. The high stomatal index and palisade ratio of P. guajava leaves reflect efficient gas exchange and photosynthesis capacity, which might be in consonance with high levels of production of secondary metabolites. Based on the present phytochemical, antioxidant, and molecular docking findings[48][49], a hypothesis can be proposed that Psidium guajava plus Cynodon dactylon synergistically augments antioxidant and antidiabetic potentials. This in vitro and in silico synergy highly advocates for the potential development of a natural therapeutic drug against oxidative stress and diabetes control.

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