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IN VITRO BIO-MEDICAL STUDIES ON *PSIDIUM GUAJAVA* LEAVES

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Abstract

Distinctive parts of guava *Psidium guajava* L. (PG) are a great wellspring of strong and intense bio medications. The present investigation was intended to extract and to identify the bioactive component of PG leaves, utilizing HPLC and assessed the activity of ethanol(70%) extract against *Helicobacter pyroli*, tuberculosis, diabetic (Alpha-glucosidase inhibitory %), arthritic (albumin denaturation inhibition) and aging (Anti-Collagenase) factorials. The outcomes of HPLC identification phenolic compounds showed that ethanolic extract contained gallic acid, caffeic acid, ferulic acid, cinnamic acid and they have highest values (4.71, 4.37, 3.82, 3.55 and 3.49µg/mg, respectively followed by resorcinol, chlorogenic, syringic acid and resormarinic acid (3.09, 2.93, 2.85 and 2.49µg/mg, respectively), while flavonoids were quercetin, hesperetin, kaempferol, quercitrin and rutin, where they presented 8.94, 7.61, 7.55, 7.13 and 6.37 µg/mg, respectively followed by catechin (5.12µg/mg) and apigenin (4.83 µg/mg). The identified alkaloids were corilagin, kaempfertin, and isoquinoline and they have values 2.13, 1.89 and 1.24µg/mg, respectively. Evaluation of inhibitory effect of PGL extract stated that PGL scored the highest values for effective concentrations (125 µg/mg) and MIC_{90} (26.6) comparing with activity of clarithromycin (C) (1.95 µg/mg and MIC_{90} (0.7), respectively against *Helicobacter pyroli* activity and 7.81µg/mg and MIC_{90} 11.94 comparing with isoniazid standard (IS) at concentration 0.24µg/mg and MIC_{90} 0.4 against tuberculosis activity. The IC_{50} of PGL against alpha-glucosidase activity comparing with values of Acarbose (A) showed that inhibitory percentage was lower at 1000µg/mg (79.22) than the value of acarbose (90.10) at the same concentration, while IC_{50} of PGL was higher (46.6) than that of acarbose (30.57). Percentage of albumin denaturation inhibition at 1000 µg/mg of both PGL extract and DSS were 71.34 and 89.35µg/mg, respectively, while IC_{50} of both were 50.26 and 15.12, respectively. Collagenase inhibition percentage of PGL recorded 82.34 but EGCGrecorded 93.24 at concentration 1000 µg/mg. The IC_{50} of PGL was higher (105.3) than the value of EGCG (40.3).

Key words : Anti-*Helicobacter pyroli*, anti-tuberculosis anti-diabetic, anti-arthritic, anti-aging and guava.

Introduction

Guava (*Psidium guajava* L.), or, in other words, a customary medication is found in nations with hot atmospheres, for example, South America, Europe, Africa, and Asia as reported by Gutiérrez, Mitchell *et al*. (2008). Leaves of *P. guajava* have a background marked by use as a conventional prescription in nations, for example, Taiwan, Japan, China and Korea as revealed by Díaz-de-Cerio, Verardo *et al*. (2015).

H. pylori contamination has been ensnared in the improvement of gastric malignancy, a multifactorial malady and a main source of mortality. The hazard factors for gastric growth have been appeared to incorporate natural factors and factors that impact host-pathogen connection and in addition the mind-boggling transaction between these variables, this meaning was supported by Zhang, Zhang *et al*. (2017). Modern lifestyle, high feelings of anxiety, smoking, and unnecessary liquor utilization, dietary lacks, and delayed utilization of non-steroidal calming drugs (NSAIDs) are among the most important etiological ecological components as showed before by Sharifi-Rad, Fokou *et al*. (2018). This bacterial contamination has been connected to the inception of incessant gastritis that could later prompt adenocarcinoma of the digestive tract as stated by Sipponen and Marshall (2000). However, it may, a few systems have been proposed to speak to the contribution of *H. pylori* disease in tumor genesis. A few

bacterial destructiveness factors, for example, the cytotoxin-related quality A (CagA) protein, present in the DNA addition component Cag pathogenicity island (CagPAI), were observed to be of noticeable significance in carcinogenesisaccording to the data of Park, Forman *et al*. (2018).

Mycobacterium tuberculosis, a facultative intracellular organism having a place with the *M. tuberculosis* complex, is the most vital reason for tuberculosis (TB) in people. In addition to *M. tuberculosis*, different individuals from the *M. tuberculosis* complex that can cause tuberculosis in people incorporate *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, and *Mycobacterium canetti* these reported before by Aro, Dzoyem *et al*. (2015). Tuberculosis, an old yet emerging in-divisive ailment, is one of the main sources of human grimness and mortality as shown by Nguta, Appiah-Opong *et al*. (2015). The alarming rise of multi-tranquilize safe (MDR), widely medicate resistant (XDR) and right now, thoroughly sedate resistant (TDR) *M. tuberculosis* strains, which are hard to control with the at present accessible fundamental enemy of tubercular medications available and the expanded occurrence of TB related with viral contamination such as HIV, have as of late muddled the chemotherapeutics of tuberculosis as stated before by Daletos, Kalscheuer *et al*. (2015).

Diabetes mellitus (DM), a standout amongst the most widely recognized metabolic scatters around the world, is expanding. In 2013, 382 million grown-ups worldwide had diabetes, and 592 million are anticipated to be influenced by 2035 as reported before by Guariguata, Whiting *et al*. (2014). The metabolic disorder has diverse parts, for example, stomach heftiness, impeded glucose digestion, dyslipidemia and hypertension, which synergistically increment the peril of cardiovascular sickness and additionally diabetes, this being obviously associated with untimely mortality. Pre-diabetes is viewed as a fundamental etiology of metabolic disorder, portrayed by a mix of overabundance muscle versus fat and insulin obstruction, and showed by hindered fasting glucose and additionally impeded glucose resistance, along these lines bringing about hyperglycemias revealed by Grundy (2012). The essential focus of hyperglycemia seems, by all accounts, to be the endothelial cells, which may actuate endothelial brokenness and quickened atherosclerosis. Herder, Dalmas *et al*. (2015) stated that these procedures are related to the advancement of a vascular incendiary reaction, with the inclusion of a few go-betweens, including receptive oxygen metabolites, chemokines and master provocative cytokines, which are unmistakably in charge of the cardiovascular inconveniences that are the main source of dismalness and mortality related with diabetes.

Rheumatoid arthritis (RA) is an immune system sickness that assaults the joints of the body and causes constant inflammation in the synovium. Inflammation in RA joints is an exceptionally mind-boggling process and includes the cooperation of an assortment of inflammatory cells, auto-antibodies, and cytokines this was documented by McInnes and Schett (2011). Conventional medications that have been used to conquer joint inflammation and restrain the advancement of RA include the illness altering hostile to rheumatic medications (DMARDs) as reported before by Conditions (2009). Traditional prescription utilizing plant separates keeps on giving wellbeing inclusion to more than 80% of the total populace, particularly in the developing world. Organization (2002) recently, the utilization of plant extracts for joint pain treatment is being advanced in the USA, particularly after the withdrawal of FDA-affirmed calming drugs. One of the plants that can possibly be produced as an anti-arthritic sedate is the guava plant (*Psidium guajava*). Guava contains tannins, phenolic compounds, flavonoids, volatile oils, sesquiterpenes and triterpenoids as investigated by Porwal *et al*. (2012). Flavonoids and phenolic compounds contained in guava have been proven to be antioxidant and anti-inflammatory where, Barbalho *et al*. (2012) found that.

Schlotmann *et al*. (2001) showed that the procedure of skin aging has been isolated into two classifications: Intrinsic and extraneous maturing Inherent skin aging or common maturing is caused by changes in the versatility of the skin after some time. Extraneous skin aging is predominately a consequence of introduction to sunlight based radiation (photoaging) as stated before by Aslam *et al*. (2006). UV presentation makes physical changes the skin because of adjustments that happen in the connective tissue by means of the development of lipid peroxides, cell substance and catalysts where, Lee *et al*. (2000) found that. It is a natural complex process, affected because of the contribution of both inherent, (for example, hereditary, hormonal and digestion changes) and outward (especially ultraviolet A and B radiation from the sun) factors. These factors prompt a decay of the skin structure, its appearance (like wrinkles, pigmentation, and changes in thickness of skin and so on.) and work. In fact, the logical comprehension of the maturing procedure empowered new test systems to be created and connected to regular item examine. Thus, hostile to maturing property of plant extracts would now be able to be evaluated by restraint of particular (key) catalysts (biomarkers), particularly elastase, hyaluronidase, and network

metalloproteinase (MMP's), which are associated with the biochemical procedures/pathway, Inhibition of collagenase movement assumes an essential job in securing the uneven turn over or fast breakdown of collagen in human aggravated or UV irradiated skin. As of late, plants have been generally examined and found to have hostile to collagenase and against elastase activities as documented in the study by Meinke *et al*. (2010). The utilization of cell reinforcements on the skin is likewise an imperative procedure to avert harm disturbed by oxidative pressure as reported in the study by Pinnell (2003). A high grouping of cancer prevention agents in healthy skin details permits the infiltration of the epidermis and dermis. The fundamental preferred standpoint of the topical organization when contrasted with the oral organization to treat and anticipate skin conditions is the immediate conveyance of bioactive substances to the objective zone, consequently wiping out worries about foundational dissemination as stated by Meinke *et al*. (2010) in their study.

The goal of thisstudy is the identification of *Psidium guajava* L. leaves extract for polyphenolic, flavonoids and alkaloids components by HPLC and assessed the activity of ethanol 70% extract on *Helicobacter pyroli*, Anti-tuberculosis, Anti-diabetic, anti-arthritic and Anti-aging activities.

Materials and Methods

Plant material

Guava (*Psidium guajava* L.), leaves were assembled from Salhia ranches, Sharkia, Egypt. The leaves were dried for four days at room temperature (20-30°C). Thereafter the dried leaves were crushed to a fine powder.

Preparation of *Psidium guajava* L. leaves extract

The dried powder of leaves test was separated by maceration with ethanol 70% (60 g dried powder into 600 ml of 70% ethanol) for 24 hr. at room temperature. In the wake of blending, the recuperated filtrate was dried in a rotational evaporator for 30 minutes at 40°C. The yield of concentrates was then lyophilized and put away at -40°C in shut compartments until required.

HPLC analysis of *Psidium guajava* L. leaves extract

HPLC investigation was performed on HPLC (Agilent Technologies, Waldbronn, Germany) device with a vacuum degasser, autosampler, a double pump GBC LC 1110 and an indicator GBC UV/vis were utilized for the chromatographic assurance. Phenolic and flavonoids segments were disengaged by using KROMASIL segment (4.6 ×150 mm, 1.8 mm molecule estimate) working at 25°C and a stream rate of 0.8 mL/min. what's more, 1 mL/min., individually) (Berek and Tarbajovská, 2002) the versatile stages utilized were methanol : water : tetrahydrofuran with acidic corrosive (23:75:1:1%) (Phase An) and acetonitrile (stage B) for phenolic parts and acetonitrile: water: formic corrosive (85:14:1) and acetonitrile (stage B) for flavonoids intensifies the separated components were observed in grouping first with GBC U.V/vis at 280 and 356 nm.

Conditions for alkaloids discovering HSC -18,3 µgm particle size (50×4.6 nm I.D) column, Mobile phase : acetonitrile, detection :UV set at 290 nm, Flow rate 1.0 ml/min, injection :20µL. The sequence of the eluted standard was 50 µg/ml (Omer, 2013).

Asses of anti-*Helicobacter pylori* Activity and (MIC_{90}) of PGL

Antibacterial activity of guava leaves extracts against *Helicobacter pylori* was determined by micro-well dilution methods. The inoculum of *Helicobacter pylori* was prepared and the suspension to 10^6 CFU/mL. The extract under investigation and the standard drug (clarithromycin) were prepared in dimethyl sulfoxide (DMSO) and subsequent tow fold dilutions (1000-0.03 µg) were performed in a 96- well plate. Each well of the microplate included 40 µl of the growth medium (brain heart infusion (BHI) plus 10% fetal bovine serum (FBS), 10 µL of inoculum and 50 µl of the diluted extract. The clarithromycin and DMSO are used as the positive and negative control, respectively. The plates were incubated at 37°C for 3 days, in 5% O_2, 10% CO_2 and 85% N_2 atmosphere. After that, 40 µL of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) at a final concentration 0.5 mg/ml freshly prepared in water was added to each well and incubated for 30 min. the change to the purple color indicated that bacteria were biologically active. The inhibition percentage was calculated using the given formula:

% inhibition = Abs Control – Abs Sample × 100/Abs Control

The concentration of samples (inhibitors) required for 90% of inhibition (MIC 90) was determined from corresponding dose-response curves. The MIC was taken to the lowest concentration, where no change of color of MTT was determined using an automatic ELISA microplate reader at 620 nm. The MIC values were done in triplicate.

Anti-tuberculosis activity of PGL

The anti-mycobacterial action of guava leaves extract was assessed against *Mycobacterium tuberculosis*

(RCMB 010094-9) utilizing the microplate Alamar blue measure (MABA) as depicted by Collins and Franzblau (1997).

Anti-diabetic activity of PGL (α-glucosidase inhibitory activity)

The α-glucosidase inhibitory activity was done by the standard strategy with a minor change as recommended by Shai *et al*. (2011).

Anti-arthritic activity of PGL

The activity was performed using albumin denaturation following to the method of Singh and Sharma (2015) with few modifications.

The inhibition percentage of albumin denaturation was calculated as follow :

% Inhibition of protein denaturation = 1-[A1/A2]100

Where, A 1 = Absorbance of control

A 2 = Absorbance of test/standard example with albumin arrangement

The IC_{50} esteem was characterized as the fixation to repress half of the protein denaturation under the measurement conditions.

Anti-aging (Anti-Collagenase) activity of PGL

Before screening in all measures, spectra for all concentrates were recorded on a Cary 300 UV-unmistakable spectrophotometer to check for impedance and moves in the lambda max. The method of asses was utilized depended on spectrophotometric techniques recommended by Thring *et al*. (2009) with a few adjustments for use in a microplate peruse.

The inhibition percentage was calculated according to the following formula :

The percentage of collagenase inhibition (%)

$$=\left(1-\frac{S}{C}\right)\times 100$$

Where, 'S' is the corrected absorbance of the samples containing collagenase inhibitor (the enzyme activity in the presence of the samples), and 'C' is the corrected absorbance of controls (the enzyme activity in the absence of the samples).

The IC_{50} value was defined as the concentration of the sample to inhibit 50% of collagenase under the assay conditions.

Statistical analysis

The results are conveyed as means ± SD (Standard Deviation). All tests were performed in triplicate and reiterated no under three times. Quantifiable complexity between bundles was controlled by one-way examination of progress (ANOVA). Ap-esteem < 0.05 was considered statistically significant.

Results

Identification and quantification of different phenolic acids, flavonoids and alkaloids in *Psidium guajava* L. (PGL) µg/mg leaves ethanol 70% extract

The HPLC chromatogram of ethanol 70% extract of *Psidium guajava* L demonstrated that identified phenolic compounds were gallic acid, resorcinol, chlorogenic acid, caffic acid, coumaric acid, ferulic acid, cinnamic acid, resormarinic acid and syringic acid as showed in (table 1) among them gallic acid, caffic acid, coumaric acid, ferulic acid and cinnamic acid have highest concentration 4.71, 4.73, 3.82, 3.55 and 3.49 µg/mg, respectively followed by resorcinol (3.09µg/mg), chlorogenic acid (2.93µg/mg) and syringic acid (2.85 µg/mg). Results in table 1 revealed that flavonoids compounds were 7 identified compounds catchin, kaempferol, rutin, quercetin, hesperetin, apigenin and quercitrin among them quercetin, hesperetin, kaempferol, quercitrin and rutin recorded highest concentration (8.94, 7.61, 7.55, 7.13 and 6.37 µg/mg), respectively followed by catchin (5.12 µg/mg) and apigenin (4.83 µg/mg). Also, table 1 results showed three compounds of alkaloids were identified, which are kaempfertin (1.89µg/mg), isoquinoline (1.24µg/mg) where corilagin had the highest concentration (2.13µg/mg). It can be noticed that flavonoids presented the highest fraction of PGL ethanolic 70% extract, especially quercetin (8.94µg/mg) which known as a high antioxidant agent.

Anti-*Helicobacter pylori* activity (% *Helicobacter pylori* inhibitory) of *Psidium guajava* L. (PGL) µg/mg leaf ethanol 70% extract

The (% *Helicobacter pylori* inhibitory) extend from 21.34 ±1.2 at concentration 0.24µg/mL to 100 ±2.5% at concentration 125 µg/mL for guava leaves ethanol extract and 81.35±1.5 to 100±0.00% for clarithromycin at concentrates extended from 0.24 to 1.94 µg/mL. MIC_{90} were 26.6 and 0.7 for guava extract and clarithromycin, respectively (tables 2).

Anti-tuberculosis activity (TB) of *Psidium guajava* L. (PGL) µg/mg leaves ethanol 70% extract

The Anti-mycobacterial action of *Psidium guajava* L. extract was given in (table 3). The outcomes indicated most activity against the *M. tuberculosis* (TB Inhibitory %) 100±0.00, at concentration 7.81µg/mL of PGL, but of IS was 100 inhibitory percentage at concentration 0.24

Table 1 : HPLC analysis data of phenolic, flavonoid and alkaloids compounds in *Psidium guajava* L. (PGL) µg/mg leaf ethanolic 70% extracts.

		PGL Concentration (µg/mg)
Phenolic compounds	Gallic acid	4.71
	Resorcinol	3.09
	Chlorogenic acid	2.93
	Caffeic acid	4.37
	Coumaric acid	3.82
	Ferulic acid	3.55
	Cinnamic acid	3.49
	Resormarinic acid	2.49
	Syringic acid	2.85
Flavonoid compounds	Catechin	5.12
	Kaempferol	7.55
	Rutin	6.37
	Quercetin	8.94
	Hesperetin	7.61
	Apigenin	4.83
	Quercitrin	7.13
Alkaloids compounds	Kaempfertin	1.89
	Isoquinoline	1.24
	Corilagin	2.13

Table 2 : Inhibitory percentage and MIC_{90} of PGL ethanolic extract comparing with values of clarithromycin (C) against *Helicobacter pylori* activity.

Concentration (µg/mL)	C	PGL
	Helicobacter pylori inhibitory (%)	
125	100±0.00	100±2.5
62.5	100±0.00	93.25±0.58
31.25	100±0.00	82.34±0.72
15.63	100±0.00	73.14±0.63
7.81	100±0.00	66.32±1.2
3.9	100±0.00	60.38±1.3
1.95	100±0.00	54.28±1.2
0.98	92.45±1.2	49.31±2.5
0.48	87.65±0.58	32.25±0.63
0.24	81.35±1.5	21.34±1.2
0	0±0.00	0±0.00
***MIC_{90}**	0.7	26.6

Table 3 : Inhibitory percentage and MIC_{90} of PGL ethanolic extract comparing with values of isoniazid standard (IS) against anti-tuberculosis activity (TB)

Concentration (µg/mL)	IS	PGL
	TB Inhibitory (%)	
31.25	100±0.00	100±0.00
15.63	100±0.00	100±0.00
7.81	100±0.00	100±0.00
3.9	100±0.00	93.25±2.5
1.95	100±0.00	86.34±1.5
0.98	100±0.00	71.28±0.58
0.48	100±0.00	59.38±0.63
0.24	100±0.00	38.14±2.1
0.12	92.35±0.58	21.07±1.5
0.06	86.34±1.5	10.69±0.36
0	0±0.00	0±0.00
MIC_{90}	0.4	11.94

Table 4 : Inhibitory percentage and IC_{50} of PGL ethanolic extract comparing with values of Acarbose (A) against alpha-glucosidase activity (as diabetic parameter).

Concentration (µg/mL)	A	PGL
	Alpha-glucosidase inhibitory %	
1000	90.10±0.58	79.22±1.5
500	86.34±1.20	74.51±2.1
250	71.34±1.50	68.72±1.2
125	63.42±2.10	62.41±0.58
62.5	60.14±0.72	58.41±1.3
31.25	50.31±1.50	41.85±0.72
15.63	43.28±1.20	36.13±2.1
7.81	32.15±0.58	29.53±1.3
0	0±0.00	0±0.00
***IC_{50}**	30.57	46.6

µg/mL. The MIC_{90} value of PGL was 11.94, while it was 0.4 of IS. These results were illustrated in table 3.

Anti-diabetic activity (Alpha-glucosidase inhibitory %) of *Psidium guajava* L. (PGL) µg/mg leaves ethanol 70% extract

The *Psidium guajava* ethanol 70% extract uncovered a noteworthy inhibitory activity of the alpha-glucosidase enzyme. The percentage inhibition at 7.81 – 1000 µg/mL convergences of *Psidium guajava* separate demonstrated a dosage subordinate increment in rate hindrance. The rate restraint changed from 29.53±1.3-79.22 ±1.5% for the most astounding focus to the least fixation this was close to Acarbose (An) as standard the

Table 5 : Inhibitory percentage and IC_{50} of PGL ethanolic extract comparing with values of diclofenac sodium standard (DSS) drug against protein denaturation.

Concentration (µg/mL)	DSS	PGL
	Inhibition of protein denaturation (%)	
1000	89.35±0.58	71.34± 1.5
500	84.12±1.2	62.58± 2.1
250	76.52±0.63	60.14± 1.5
125	70.14±0.58	58.34± 1.2
62.5	68.28±0.63	51.39±2.1
31.25	59.14±1.2	47.84±1.5
15.63	51.21±0.58	31.24±1.3
7.81	31.12±1.2	16.85±1.5
0	0	0
***IC_{50}**	15.12	50.26

Table 6 : Inhibitory percentage and IC_{50} of PGL ethanolic extract comparing with values of Epigallocatechin gallate EGCG against aging marker (Collagenase activity as aging parameter).

Concentration (µg/mL)	EGCG	PGL
	Collagenase inhibition %	
1000	93.24±1.78	82.34±1.2
500	84.22±1.3	73.25±1.2
250	69.35±0.58	66.17±0.63
125	58.98±1.5	53.97±2.5
62.5	53.21±1.2	41.14±1.2
31.25	48.69±0.63	22.14±0.58
15.63	32.55±0.58	16.31±1.3
7.81	19.34±2.5	6.31±0.72
0	0±0.00	0±0.00
***IC_{50}**	40.3	105.3

outcomes were gone from 32.15 ±0.58 to 90.10 ±0.58 at same concentrates (table 4).

Inhibition of protein denaturation (%) by *Psidium guajava* L. (PGL) µg/mg leaves ethanol 70% extract

The consequences of *in vitro* denaturation of egg albumin of *P. guajava* L. condensed in (table 5) guava leaves extract demonstrated an inhibition in protein denaturation with an IC_{50} estimation of 50.26 µg/mL (IC_{50} estimation of Diclofenac sodium is 15.12 µg/mL). As appeared, *P. guajava* L. had demonstrated hindrance of protein denaturation closest to Diclofenac sodium showing that *P. guajava* L. is an intense enemy of ligament operator. The greatest level of hindrance was communicated at 1000 µg/mL.

Anti-aging (Collagenase inhibitory %) of *Psidium guajava* L. (PGL) µg/mg leaves ethanol 70% extract

The incubation of the enzyme with the concentrate fundamentally inhibited the protein. A grouping of 1000 µg/mL extract indicated 82.34 ±1.2% catalyst hindrance, while bring down convergences of 7.81, 15.63, 31.25 and 62.5 µg/mL caused 6.31±0.72%, 16.31 ±1.3%, 22.14 ±0.58% and 41.14 ±1.2% protein enzyme inhibition, respectively. These outcomes were compared with Epigallocatechin gallate the outcome at high concentrate 1000 µg/mL was 93.24±1.78% though bring down groupings of 7.81, 15.63, 31.25 and 62.5 µg/mL standard caused 19.34 ±2.5%, 32.55 ±0.58%, 48.69 ±0.63% and 53.21±1.2% enzyme inhibition, respectively (table 6).

Discussion

Guava leaves extracts are a potential wellspring of common cell reinforcements as showed by results obtained by Ojan and Nihorimbere (2004), who stated that these cell reinforcement properties are related to its phenolic compounds, for example, ferulic acid, quercetin, guavin B, ascorbic acid, Gallic corrosive and caffeic acid these results were supported by Thaipong *et al*. (2005). The phenolic profile of Spanish guava leaves has as of late been accounted for with an appraisal of the grouping of various flavonoids. Among these, flavonols and flavan-3-ols were the real subclasses found in the Andalusia guava leaves as stated by Díaz-de-Cerio *et al*. (2016), who revealed thatmain phenolic segments beforehand recognized in the product of *Psidium guajava* are glycosides of myricetin, quercetin, kaempferol, luteolin, apigenin and isorhamnetin, a B-type proanthocyanidin (gallocatechin-catechin) and two benzophenone mixes stated recently by Rojas-Garbanzo *et al*. (2017). Results in this study were in harmony with those reported above attributed to the highest effect of these compounds as an antioxidant agent.

Guava leaves extract give has well extreme against *Helicobacter pylori* and as Anti-tuberculosis. Anti *H. pylori* Potency of *Psidium guajava* L. leaves was IZD = 33.0 ± 2.3 mm (240 µg/circle) as reported by Salehi *et al*. (2018). The numbers of synergetic interactions between PGL against *M. tuberculosis* are few. In this way, more examination is required into the system of activity of flavonoids. Tulin (2015) demonstrated that quercetin is a piece of the 3-hydroxy gathering, which enhances antimycobacterial activities and synergistic cooperation. Kaempferol likewise has a functioning

compound required beside the hydroxyl gatherings in ring B, giving potentiation activity of flavonoids.

Guava leaves extract indicates anti-diabetic action, the *in vitro* activities of α-glucosidase proteins in guava extract was a polymerized polyphenol. Moreover, polysaccharides from guava leave additionally displayed α-glucosidase hindranceas revealed by Zhang *et al*. (2016).

The IC_{50} of guava leaves extract to inhibition of protein denaturation was near to diclofenac sodium standard (DSS). The provocative reaction happens when cells and body tissues are harmed by natural, chemical, or physical upgrades, for example, microorganisms, injury, poisons, or warmth. It is a standout among the most imperative safeguard components, or, in other words, evacuation of the harmful improvements and commencement of the recuperating procedure. Macrophages are entered players in different fiery illnesses and in the resistant reaction where they discharge pro inflammatory middle people and proteins, including interleukin-6 (IL-6), tumor corruption factor-a (TNF-a), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) as stated by Grip *et al*. (2003). Plant extracts have been utilized as a wellspring of pharmaceuticals for a wide assortment of a human issue. Homegrown and characteristic items have as of late gotten expanded consideration in view of their organic and pharmacological activities as stated by Eze *et al*. (2017). *Psidium guajava* L. leaves have been utilized for the treatment of stiffness, fever, joint pain and other provocative conditions as found (Kim *et al*., 2015) in their study. Quercetin may add to mitigating action as mentioned by Metwally *et al*. (2010). Flavonoids have natural activities, for example, antioxidant, anti-aging, hostile to cancer-causing, mitigating, against atherosclerosis, cardiovascular insurance and change of endothelial capacity, the hindrance for angiogenesis and cell multiplication activities as stated by Rahman *et al*. (2015) in their study. Phenolic mixes add to pain relieving, calming, against microbial, hepatoprotective and cancer prevention agent activities (Choudhury *et al*., 2012).

The inhibitory effects on collagenase activities showed that 82.34±1.2% ethanol PGL extract ata concentration of 1000µg/mL possessed moderate inhibition comparable to that of 93.24±1.78% Epigallocatechin gallate at the same concentration. The effect of PGL as anti-aging may be due to its polyphenolic components. Sin and Kim (2005) inspected the inhibitory activities of different flavonoids, including the flavanones, flavones/isoflavones and flavonols, on collagenase from Clostridium histolyticum to build up their helpful potential against skin aggravation and photoaging. As a rule, the flavonols were more grounded inhibitors than the flavones/isoflavones, and this showed the significance of the C-3 hydroxyl substitution. Quercetin was the most dynamic flavonoid the IC_{50} was 286 microM. This work needs to study the action of guava leaves extract on therapeutic activities under-considered especially *Helicobacter pyroli*, anti-tuberculosis and anti-aging.

Conclusion

In conclusion, *Psidium guajava* L. leaves as cheap raw material contains high amount from Gallic acid, Quercetin and Corilagin according to HPLC analysis these compounds play a great role as antioxidants activities. The ethanol 70% extract of the plant gave high possess inhibition for *Helicobacter pyroli*, Anti-tuberculosis, Anti-diabetic, anti-arthritic and Anti-aging, PGL will be accepted to be more adequate by the human body, while reducing the risk of using synergistic drugs.

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