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Insight on the fractionations and structural characterizations of innovative antidiarrheal compounds screened from leaves of *Psidium guajava* of local origin in Pakistan

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Abstract: Numerous ailments have been effectively treated with natural plants for long time all over the world. Plants provided a back bone for the exploration of novel medicinal compounds. Therefore, chief focus of our study was to isolate the biologically active compounds from the plant source and evaluate their antidiarrheal potentials, as diarrhea is still the most dominant disease in developing countries. The isolation and structure elucidation of two new compounds were identified from methanolic and chloroform extracts of *Psidium guajava* (guava) leaves. Extracts of plants were acquired by successive maceration from dried powder. Castor oil induced diarrheal-model was used to evaluate the antidiarrheal activity and therapeutic response was endorsed to the suppression of normal and wet stools in Spraug Dawley rats. Through the series of fractionations, compound-A was obtained from methanolic extract and named 3-(4-amino 1,3,8-tri-OH 5,6-di-CH_3 7-propyl 1,2,3,4,4a,5,8,8a-octahydronaphthalen 2-yl) propanoic3-(4-NH_3 7-butyl 1,3,8-tri-OH 5,6-di-CH_3 1,2,3,4,4a,5,8,8a-octahydronaphthalen 2-yl)propanoic anhydride. Compound-B was entitled 5-(3-hydroxy-1,4-di-CH_3-1,2,3,4,4a,5,8,8a-octahydronaphthalen-2-yl)pent-3-enoic acid was acquired from the chloroform extract. The structure elucidations of both compounds were interpreted through spectroscopic data, including EI-MS, FTIR, ^{1}HNMR and ^{13}C-NMR. The significant antidiarrheal activities were determined with crude extracts and isolated compounds. In inference, present study revealed that substantial antidiarrheal feature of guava is confined to the identified compounds.

Keyword: Antidiarrheal, *Psidium guajava*, Myrtaceae, methanolic extract, spectroscopic data.

INTRODUCTION

The morbidity and mortality rate of diarrhea is still prominent in many developing countries worldwide. This deadly disease annually executed millions of people, particularly low income community, infants and children under 5-year of ages (Porwal *et al*., 2012). Diarrheal symptoms like frequent discharge of watery and semisolid feces accompanied by abdominal pain, discomfort and loss of electrolytes leading to dehydration (Longe and Dipiro, 1992). As primary health care levels, herbal drugs have been practiced to control diarrhea in more than 80% community in Asia and Africa. This conventional treatment of diarrheal disease with herbal remedies is highly appreciated and still need to explore some new medicinal compounds from natural sources (Mazumdar *et al*., 2015). *Psidium guajava* is nutritionally and therapeutically important worldwide, infusion and decoction of its leaves have been used to manage dysentery, gastritis and pain in Latin America and Colombia (Leonti *et al*., 2001, Lufuluabo *et al*., 2018). Leaves of guava also employed as anti-diabetics, antihypertensive, antiseptic, antidiarrheal and wound healing in Africa, Philippines and China (Smith *et al*., 2018). In Pakistan, Uruguay and USA guava shown an effective management of inflammation, colic pain, leucorrhoea and bacterial infection (Gutiérrez *et al*., 2008). On the consequence of guava's significance, it was chosen for our investigation for antidiarrheal activity and structural characterization of two innovative compounds. As concerned the choice of selection of leaves particularly, the astonishing amount of secondary metabolites produced (leaves) in month of March-April during spring session in Pakistan. The family of *Psidium guajava* L. is Myrtaceae included genera around 133 and species above 3800. Guava is native in Central America and Mexico and currently extensively cultivated in tropical region like Pakistan, Bangladesh, India and West Indies (Gutiérrez *et al*., 2008).

The antidiarrheal activity of *P. guajava* has been stated from its alcoholic and aqueous crude extracts. Our works evaluate the significant antidiarrheal potential of mehtanolic (PG-M) and chloroform (PG-C) extracts in leaves of the plant. In addition, assessment of the significant antidiarrheal activity of isolated and purified compounds (compound-A & compound-B) from PG-M and PG-C was also performed. The purification and

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isolation of new compounds were achieved by different chromatographic techniques and ultimate structure elucidations were attained through various spectroscopic methods. Various bioactivities have been stated in guava extracts that associated with the secondary metabolites produced in plant (Gutiérrez *et al*., 2008). The current studies focused on the isolation and structural interpretation of new antidiarrheal compounds from guava leaves, because of its therapeutic importance and easily access by the global community.

MATERIALS AND METHODS

Collection and extraction of plant

Collection of *Psidium guava* L. (guava) leaves was done from City Garden near Sialkot International Airport, Province of Punjab, Pakistan. Leaves were shade dried for the period of fortnight after taxonomical identification from Dr. Sultan Ahmed Herbarium, Botany Department, Government College University Lahore, Pakistan through specimen # GC - Herb - Bot - 2408. The course powder (1000 g) was macerated through successive maceration with 5.5-liter chloroform for the period of one week. Filtrated was concentrated by employing rotary-evaporator (IKA-HB10-Germany) and residue was shade dried for 48 hours and weighed (968 g). Then Residue was macerated with methanol (5 L) and dried gummy mass was obtained by applying rotary-evaporator. The resulting percentage yields (w/w) of both crude extracts (gummy masses) were 2.32% and 22.68% of chloroform and methanol respectively. Crude extracts were stored in amber color glass bottles at 04˚C.

Animals used for the study

The procurement of Sprague-Dawley rats (200±50 g) was made from animal house, Faculty of Pharmacy, University of Lahore, Pakistan. Standard laboratory environment was provided continuously and pallet diet was given routinely for specified times as well as *ad libitum* access (water) was provided to them. All animals were treated according to the US guidelines (NIH-publication #85-23-Revised 1985). The approval of bio-assay protocols and procedures were organized through Faculty of Pharmacy, University of the Lahore Animal Ethical Committee (No.IAEC-2016-17, dated: 25/08/2016) and strictly followed the rules and regulations (Shabbir *et al*., 2016).

Suppression of castor oil-induced diarrhea with crude extracts and isolated novel compounds

The evaluation of antidiarrheal activity of methanolic and chloroform extracts was determined through castor oil-induced diarrheal model (Suleiman *et al*., 2008). The evaluation parameters like; total number of normal and wet feces was recorded. Four groups of rats were made randomly comprising 06 animals each (n=6) in pre-cleaned stainless steel cages. White plastic sheets were placed at the bottom of all cages for the collection and counting of feces. First 02 groups were entitled test group, whom given 100mg/kg (body wt. of rat) of PG-M and PG-C extracts in 10% DMSO orally with oral gavage. While, 3rd group was –ve control who received only 10% DMSO and 4th one was +ve control who fed Lopramide (2 mg/kg) in DMSO by mouth. After around 30 min of dosing with extracts and standard drug, diarrhea was induced with castor oil (10ml/kg) in all groups. The dropping of wet and dry stools were monitored for 04 hours (Holowacz *et al*., 2016; Mobashar *et al*., 2019). The assessment of antidiarrheal activity of isolated compounds (compound-A and compound-B) was determined through same protocol since, using only 10mg/kg doses of newly isolated compounds.

Toxicity of plant extract

Five groups of healthy rats were made with 25 animals. First 02 groups were administered 750mg/kg of PG-M and PG-C extracts and next 02 were given 500mg/kg of both extracts of *Psidium guajava* with oral gavage. The 5th group was kept as control who given DMSO 10% only. Animals were observed after 04, 24 and 168 hrs and no any signs of toxicity in the form of morbidity and mortality were perceived in any animal.

STATISTICAL ANALYSIS

The 5.02 version of Graph-Pad Prism was used to analyze the statistical data. The mean ± SEM was applied for presenting the data by using 06 observations in each group. The evaluation of biological data was done by One-Way ANOA beside post Tukey's multiple comparison test. The statistical significance values were set at $P\ value \leq 0.05$.

Physical and spectroscopic data of isolated compound-A

A 25mg white-crystalline compound was isolated from the methanolic extract of *P. guajava* through the series of column chromatography. The compound-A exhibited melting point at 212˚C. The FTIR $_{vmax}$ (KBr cm^{-1}): 1080, 1120, 1215, 1276, 1377, 1455, 1718, 2853, 2926 & 3390. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 500MHz), ^{13}C-NMR ($CDCl_3$, 300MHz) (table 1), EI-MS *m/z* (rel-int): 43(25), 57(46), 71(30), 111(20), 148.9(10), 316(36), 647(100), 662(76.7) & 678(16). HR-EI-MS m/z: 678$[M+H]^+$ (intended for $C_{37}H_{62}N_2O_9$:678).

Physical and spectroscopic data of isolated compound-B

A 77mg dark-brown amorphous powder fractioned from chloroform extract of *P. guajava* with 256˚C melting point. FTIR-υ_{max} (KBr cm$^{-1)}$: 963, 1120, 1271, 1377, 1461, 1517, 1634, 1723, 2853 & 2920. ^{1}H-NMR ($CDCl_3$+CD_3OD-500MHz), ^{13}C-NMR ($CDCl_3$-300MHz) (table 2), EI-MS (*m/z*) rel-int: 43(62), 57(100), 71(80), 111(37), 148.9(67), 167(29) & 279(14.4). HR-EI-MS (m/z): 278$[M+H]^+$ (calculated for $C_{17}H_{26}O_3$ 278).

Table 1: ^{1}H and ^{13}C NMR data of compound-A

Carbon #	^{1}H NMR δ(ppm)	^{13}C NMR δ(ppm)	Carbon #	^{1}H NMR δ(ppm)	^{13}C NMR δ(ppm)
C1	3.58 - 3.59d (1H)	68.1	C20	1.64 - 1.66m (2H)	30.1
C2	0	128.7	C21	2.02 - 2.04t (2H)	22.6
C3	0	129.6	C22	1.39 - 1.42m (1H)	32.6
C4	2.03 - 2.04m (1H)	31.8	C23	3.58 - 3.59t (1H)	41.3
C5	1.66 - 1.67m (1H)	26.9	C24	2.31 - 2.34t (1H)	38.6
C6	2.31 - 2.34t (1H)	38.6	C25	1.66 - 1.67m (1H)	26.9
C7	3.58 - 3.59t (1H)	41.3	C26	2.03 - 2.04m (1H)	31.8
C8	1.39 - 1.42m (1H)	32.6	C27	0	130.8
C9	3.61 - 3.64t (1H)	38.6	C28	0	130.9
C10	1.60 - 1.61t (1H)	31.3	C29	3.58 - 3.59d (1H)	68.1
C11	1.20 - 1.21d (6H)	19.6	C30	1.60 - 1.61t (1H)	31.3
C12	1.26 - 1.27d (6H)	13.9	C31	3.61 - 3.64t (1H)	38.6
C13	1.69 - 1.72m (4H)	23.6	C32	1.20 - 1.21d (6H)	19.6
C14	1.31 - 1.32m (2H)	22.6	C33	1.26 - 1.27d (6H)	13.9
C15	0.81 - 0.84t (6H)	10.8	C34	1.69 - 1.72m (4H)	29.0
C16	1.64 - 1.66m (2H)	22.6	C35	0.93 - 0.95m (2H)	29.2
C17	2.02 - 2.04t (2H)	30.2	C36	087 - 0.88m (2H)	28.8
C18	0	171.7	C37	0.81 - 0.84t (6H)	10.8
C19	0	171.7	-	-	-
OH	4.16s		NH	8.08s	
OH	4.26s		NH	7.69s	

Table 2: ^{1}H and ^{13}C NMR data of compound-B

Carbon #	^{1}H NMR δ(ppm)	^{13}C NMR δ(ppm)	Carbon #	^{1}H NMR δ(ppm)	^{13}C NMR δ(ppm)
C-1	2.30 - 2.34t (2H)	29.3	C-10	1.59 - 1.60t (1H)	38.7
C-2	7.49 - 7.52t (1H)	128.8	C-11	0.91 - 0.93d (6H)	14.1
C-3	7.67 - 7.69t (1H)	129.5	C-12	0.91 - 0.93d (6H)	10.9
C-4	2.30 - 2.30t (2H)	28.9	C-13	2.30 - 2.34t (2H)	27.1
C-5	1.59 - 1.60t (1H)	32.7	C-14	4.18 - 41.19t (1H)	132.4
C-6	1.66 - 1.68m (1H)	37.1	C-15	4.21 - 4.22m (1H)	130.9
C-7	2.32 - 2.34t (1H)	68.2	C-16	3.21 - 3.22t (2H)	22.9
C-8	1.63 - 1.64t (1H)	38.9	C-17	0	167.8
C-9	1.66 - 1.68t (1H)	31.9			
OH	1.89s		OH	8.08s	

RESULTS

Control of castor oil-induced diarrhea with Psidium guajava

Suppression of diarrhea with methanolic and chloroform extracts

The significant control of normal stool formation was recognized with methanol (91.31%) and chloroform extracts (82.62%) of *P. guajava* when compared with lopramide (95.67%) and negative control group fig. 1A. The substantial response of wet feces control (95.02%) was observed with both PG-M and PG-C extracts on comparison to control groups fig. 1B.

Antidiarrheal activity with isolated compound-A & compound-B

The evaluation of antidiarrheal activity in the form of decrease number of normal and wet feces was determined in pretreated rats with compound-A and compound-B orally. The vital suppression of normal stool drop was observed with compound-A (86.96%) and compound-B (91.31%) when compared with reference (95.67%) and control groups fig. 2A. The suppressions of wet feces were the most significant (95.67%) with both isolated compounds and almost the same % inhibition was noticed as seen with lopramide fig. 2B.

Isolation of compound-A from methanolic extract of PG-M

Filled 10g methanolic extract (PG-M) in column and fractioned through column chromatography on silica gel (60) with chloroform: methanol (80%:20%) used as mobile phase. Six fractioned were obtained and assigned specific codes; PG-M1, PG-M2, PG-M3, PG-M4, PG-M5 and PG-M6. On the manner of precise spot detection on TLC, fraction PG-M5 (113mg) was further fractionated

with gel filtration chromatography using sephadex (LH-20) and methanol as mobile phase. Resulting 03 fractions were achieved like; PG-M5A, PG-M5B and PG-M5C. The fraction PG-M5A (30mg) was selected for the purification with preparative TLC by employing 80%:20% ratio of chloroform : methanol as mobile phase and ultimately purified compound-A (25mg) was attained (fig. 3).

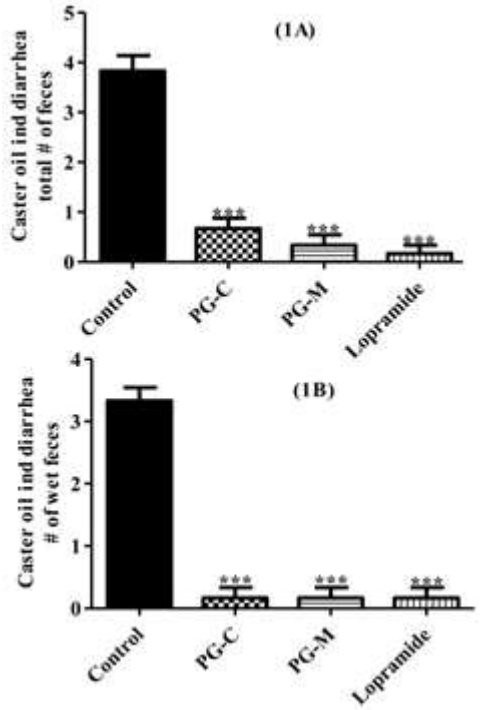

Fig. 1: Treatment with PG-C and PG-M extracts of *P. guajava* and perceived significant inhibition of diarrhea, induced with castor oil. It was observed that total # of normal feces (fig. 1A) and wet feces (fig. 1B) after 4 hr castor oil administration. Data signified the Mean ± SEM, where n = 6 *P<0.05, **P<0.01 and ***P<0.001 using 01-way ANOVA followed by Tukey's test on comparison with standard and control groups.

Isolation of compound-B from chloroform extract of PG-C

Packed 10g of PG-C crude extract in glass column and fractionated with 90%:10% ratio of $CHCl_3$: CH_3OH as mobile-phase. Five large fractions were collected as; PG-C1, PG-C2, PG-C3, PG-C4 and PG-C5 after pooling small fractions of chloroform extract of *P. guajava*. The 2nd fraction (PG-C2, 285mg) was further screened by column chromatography using sephadex (LH-20) and eluted with CH_3OH and obtained PG-C2A, PG-C2B and PG-C2C segments. The final isolation and purification of compound-B was achieved from PG-C2B (89mg) fraction through preparative TLC using chloroform and methanol ratio 80% and 20% respectively (fig. 4).

Structure-elucidation of compound-A

The FTIR spectrum of compound-A offered signal at 1718 cm^{-1} because of carbonyl functional group in the molecule, along the band at 2926 cm^{-1} showed the presence of Sp^3 C−H and at 2853cm^{-1} indicated Sp^2 C−H due to stretching vibrations. The molecular ion peak at 678 m/z value deduced the molecular formula ($C_{37}H_{62}N_2O_9$) using JEOLJMS-600H. Six alcoholic groups specified bands on ^{1}H-NMR spectra at 3.58-3.59d, 3.58-3.59t, 3.61-3.64t, 3.58-3.59t, 3.58-3.59d and 3.61-3.64t ppm values. Signals shown at δ 8.08s and 7.69 positions indicated primary-amino groups in compound-A. The ^{1}H represented the aliphatic rings at δ 2.31-2.34t, 1.66-1.67m, 1.39-1.42m, 3.58-3.59t, 1.60-1.61t, 3.61-3.64t, 2.31-2.34t, 3.58-3.59t, 1.60-1.61t, 1.66-1.67m and 3.61-3.64t in structure-A. The spectrum of ^{13}C-NMR of compound-A showed total 37 C signals such as; 16 for methane, 05 for methyl and for 09 methylene groups. The presence of carbonyl-carbons indicated at the downfield position δ 171.7. Alcoholic peaks appeared at 38.6, 41.3, 68.1, 38.6, 41.3 and 68.1 ppm locations.

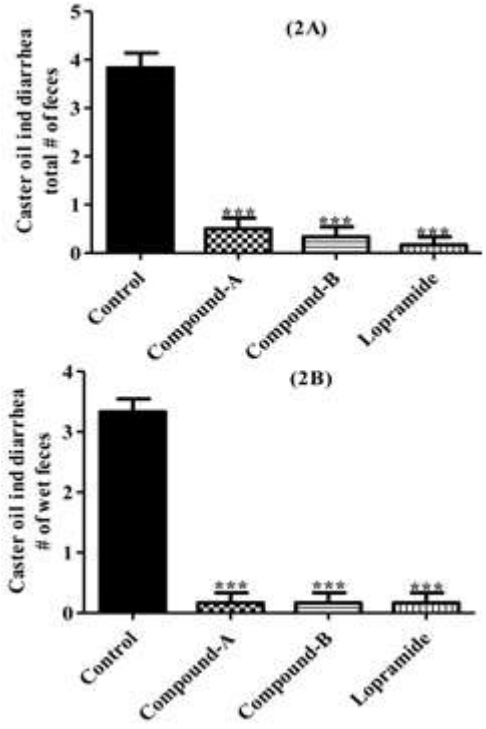

Fig. 2: Pretreatment with compound-A and compound-B, perceived substantial control of diarrheic attack. The normal (fig. 2A) and wet stool formation (Fig. 2B) were observed after 4-hour of castor oil feeding. The presented data implied the Mean ± SEM (n = 6) and *P<0.05, **P< 0.01 & ***P<0.001 by one way ANOVA followed by post-hoc Tukey test on comparison with control and reference groups.

On relating to the above congregated data, structure of the compound-A was elucidated as 3-(4-amino-1,3,8-

trihydroxy 5,6-dimethyl 7-propyl 1,2,3,4,4a,5,8,8a-octahydro naphthalene 2-yl)propanoic 3-(4-amino 7-butyl 1,3,8-trihydroxy 5,6-dimethyl 1,2,3,4,4a,5,8,8a-octahydronaphthalen 2-yl) propanoic anhydride. It was concluded that compound-A found to be a new and first time isolated from the leaves of guava with significant antidiarrheal potential (fig. 5).

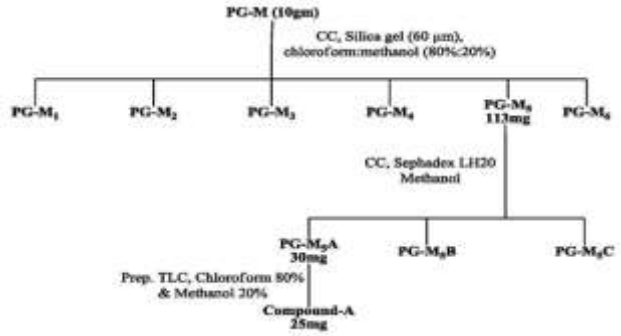

Fig. 3: Scheme of isolation of compound-A from methanolic-extract of *P. guajava*

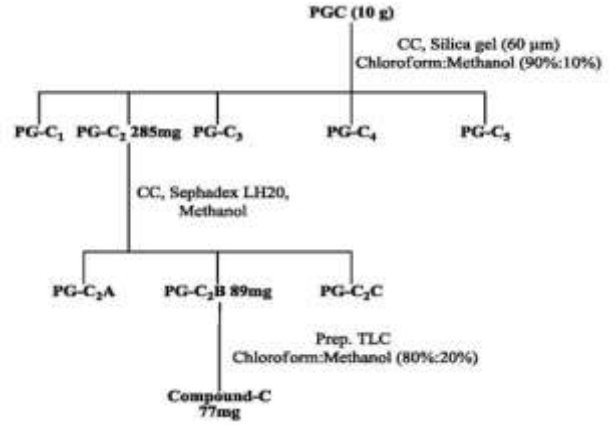

Fig. 4: Isolation scheme of compound-B from chloroform extract of *P. guajava*

Structural characterization of compound-B

The transmittance band in FT-IR spectrum at 1723cm^{-1} displayed the presence of carbonyl-functional group. The stretching vibrations at 2920cm^{-1} and 2853cm^{-1} represented the existence of Sp^3 C-H and Sp^2 C-H respectively. From the molecular-ion bar [M H] at 278 m/z value, molecular formula was assumed as $C_{17}H_{26}O_3$ using ˚JEOLJMS-600H˚. The ^{1}H-NMR spectrum of compound-B verified a peak of -OH at δ 2.32-2.34t. The $^+$H of aliphatic ring indicated at different ppm values such as; 2.32-2.34t, 1.59-1.60t, 1.63-1.64t, 1.66-1.68m, 1.59-1.60t and 1.66-1.68t. The existence of -CH_3 groups in the isolated compound was confirmed by the appearance of peaks at δ 0.91-0.93d (6H) & 0.91-0.93d (6H). As concerned the spectrum of ^{13}C-NMR, we identified total 17 signals of carbon, including 10 for methane and 02 for methyl groups, 01 for carbonyl carbon and 04 for methylene group in compound-B. The presence of C=C was established from the downfield peaks at various values of δ (132.4, 130.9, 128.8, 129.5). As per description of the above said data, the final structure of the compound-B was anticipated as 5-(3-hydroxy, 1,4-dimethyl, 1,2,3,4,4a,5,8,8α-octahydronaphthalen, 2-yl) pent, 3-enoic acid. The isolated innovative compound-B was found to be a potent antidiarrheal compound which first time obtained from *P. guajava* leaves (fig. 6).

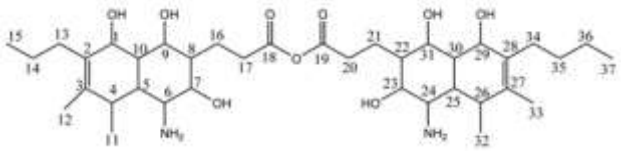

Fig. 5: Structure of Compound-A

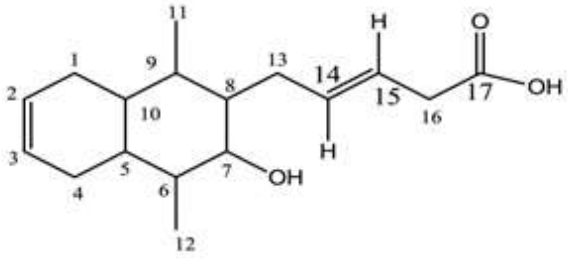

Fig. 6: Structural Formula of Compound-B

DISCUSSION

The focus of our study is to evaluate the antidiarrheal activity of methanolic and chloroform extracts of leaves of *Psidium guajava* and innovation of two anti-diarrheal compounds. The significant anti-diarrheal activity was also observed from the newly isolated compounds. The absorption and secretion of intestinal mucosa (duodenum, jejunum and ileum) played a prime role to maintain the body functions. Any malfunction of intestinal physiology in the form of diarrhea is associated with increased motility of intestinal-gut and excessive fluid loss via feces (Radha and Shrotri, 1982). Diarrhea was traditionally treated with herbal drugs that proved through various *in-vivo* investigational models (Tangpu and Yadav, 2004, Lufuluabo *et al*., 2018). Conventional plants exerted their therapeutic response in various means like; slowing gastric emptying and motility as well as stimulating gut wall to absorb fluids. Because of these vital roles of herbal plants, lot of plants could be used to control diarrhea and diarrheal related diseases (Palombo, 2006). Intestinal enzymes metabolize the castor-oil into active form (ricinoleic acid) which irritates the gastric mucosa in the form of inflammation through prostaglandin secretion. Prostaglandin then initiated the intestinal motility and mucosal secretion stemmed watery stools (Palombo, 2006). As lopramide exhibited its therapeutic effect by the suppression of intestinal secretion and motility and assumed to be categorized in the class of antidiarrheal drugs (Tangpu and Yadav, 2006). Antidiarrheal activity

with various protocols has been reported in different extracts of the leaves of *P. guajava* (Gupta and Birdi, 2015; Koriem *et al*., 2019). The castor oil-induced diarrheal (*in-vivo*) model was executed in our current study for the evaluation of antidiarrheal potential. The substantial antidiarrheal efficacy with PG-C and PG-M as well as with isolated compounds through reduction of watery and wet stool formation as well as gastric motility suppression met with standard criteria of anti-diarrheal drugs. The methanolic extract of guava exhibited substantial suppression of normal stool (91.31%) and wet stool (95.02%). The PG-C also showed significant fall (82.62%) of ordinary and diarrheic feces (95.02%). The previously reported diarrhea control model was not as effective as we perceived in our castor-oil induced diarrheal model (Lin *et al*., 2002; Ojewole *et al*., 2008). The momentous control of watery stools was also detected with new isolated compounds; compound-A showed 95.02% and compound-B exhibited 95.05% respectively. Thus our both extracts (PG-M & PG-C) and new compounds showed superior response comparative to other studies. The structure elucidation of compound-A and compound-B were accomplished by reading the detailed experimental spectrophotometric data. The resulting newly isolated; compound-A related propanoic anhydride character and compound-B belongs to acidic in nature.

CONCLUSION

The current study focused on the assessment of antidiarrheal activity of methanol and chloroform extracts of *P. guajava*. Isolation and purification of two potent natural antidiarrheal compounds were gathered from the leaves of guava through various chromatographic methods. In addition, significant antidiarrheal activities of identified compounds (new compounds) were also evaluated using castor oil induced diarrheal model. This study explored and found potent antidiarrheal compounds from the cheapest source; leaves of guava which are in easy access worldwide. However, further studies would also be recommended for the isolation and purification of more bioactive compounds from other fractions of *P. guajava* that might be lined to more powerful therapeutic compounds.

REFERENCES

Gupta P and Birdi T (2015). *Psidium guajava* leaf extract prevents intestinal colonization of Citrobacter rodentium in the mouse model. *J Ayurveda Integr Med.*, **6**(1): 50-56.

Gutiérrez RMP, Mitchell S and Solis RV (2008). *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol.*, **117**: 1-27.

Holowacz S, Blondeau C, Guinobert I, Guilbot A, Bardot V, Cotte C and Bisson JF (2016). Anti-diarrheal and anti-nociceptive effects of a hydroethanolic leaf extract of walnut in rats. *Med Aromat Plants (Los Angel)*, **5**(268): 2167-2177.

Koriem KM, Arbid MS and Saleh HN (2019). Antidiarrheal and protein conservative activities of *Psidium guajava* in diarrheal rats. *J. Integr. Med.*, **17**(1): 57-65.

Leonti M, Vibrans H, Sticher O and Heinrich M (2001). Ethnopharmacology of the Popoluca, Mexico: an evaluation. *J. Pharm. Pharmacol.*, **53**: 1653-1669.

Lin J, Puckree T and Mvelase T (2002). Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. *J. Ethnopharmacol.*, **79**(1): 53-56.

Longe R and Dipiro J (1992). Diarrhoea and constipation. In pharmacotherapy: A physiologic Approach, 2nd edn, Elsevier, **1**(2): 1-8.

Mazumdar S, Akter R and Talukder D (2015). Antidiabetic and antidiarrhoeal effects on ethanolic extract of *Psidium guajava* (L.) Bat. leaves in Wister rats. *Asian Pac J Trop Biomed.*, **5**(1): 10-14.

Mobashar A, Shabbir A and Hassan S (2019). Anti-inflammatory effects of *Trigonella gharuensis*: Comparative analysis in BALB/C mouse model. *Pak J Pharm Sci.*, **32**(5-s): 2287-2293.

Lufuluabo LG, Moke LE, Bongo GN, Liyongo CI, Ashande CM, Sapo BS, Zoawe BG and Mpiana PT (2018). A review on the Phytochemistry and Pharmacology of *Psidium guajava* L. (Myrtaceae) and Future direction. *Discov Phytomed.*, **5**(2): 7-13.

Ojewole JA, Awe EO and Chiwororo WD (2008). Antidiarrhoeal activity of *Psidium guajava* Linn.(Myrtaceae) leaf aqueous extract in rodents. *J Smooth Muscle Res.*, **44**(6): 195-207.

Palombo EA (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother Res.*, **20**(9): 717-724.

Porwal V, Singh P and Gurjar D (2012). A comprehensive study on different methods of extraction from guajava leaves for curing various health problem. *Int J Eng Res Appl.*, **2**(6): 490-496.

Radha Y and Shrotri D (1982). Comparison of antidiarrhoeal activity of some drugs in experimental diarrhoea. *Indian J. Pharmacol.*, **14**(4): 293-98.

Shabbir A, Shahzad M, Ali A and Zia-ur-Rehman M (2016). Discovery of new benzothiazine derivative as modulator of pro-and anti-inflammatory cytokines in rheumatoid arthritis. *Inflammation*, **39**(6): 1918-1929.

Smith NJ, Williams, JT, Plucknett DL and Talbot JP (2018). *Tropical forests and their crops*, Cornell University Press.

Suleiman MM, Dzenda T and Sani C (2008). Antidiarrhoeal activity of the methanol stem-bark extract of Annona senegalensis Pers.(Annonaceae). *J. Ethnopharmacol.*, **116**(1): 125-130.

Tangpu V and Yadav AK (2004). Antidiarrhoeal activity of Rhus javanica ripen fruit extract in albino mice. *Fitoterapia,* **75**(1): 39-44.

Tangpu V and Yadav AK (2006). Antidiarrhoeal activity of *Cymbopogon citratus* and its main constituent, citral. *Pharmacology online,* **2**: 290-298.

Uroos M, Abbas Z, Sattar S, Umer N, Shabbir A and Sharif A (2017). Nyctanthes arbor-tristis ameliorated FCA-induced experimental arthritis: A comparative study among different extracts. *Evid. Based Complementary Altern. Med.,* pp.1-13.