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Evaluation of Anti-Diarrhoeal and Anti-Microbial Activity of *Psidium Guajava* Leaves Extract

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ABSTRACT

Background: *Psidium guajava* is found in Nepal and is commonly known as Aamba. Aamba is evergreen tree with green to yellow fruit and with white flower. Although evergreen it only produces fruit in winter. The leaves of *Psidium guajava* is popularly used as an anti-diarrhoeal agent in Nepali communities. There is limited research on anti-diarrhoeal, phytochemical screening and anti-microbial activity of *Psidium guajava* found in Nepal.

Methods: The extracts were extracted using maceration process for 4 days. The extracted solutions (ethanolic, methanolic and water) were used to determine phytochemicals. For anti-microbial activity, five micro-organisms were used like *S. aureus*, *Klebsiella*, *P. aeruginosa*, *S. typhi* and *E. coli*. All extract and Standard drug (Norfloxacin and Amoxicillin) used for anti-microbial activity. For anti-diarrhoeal activity, 30 swiss albino mice were deprived of food for 12 hours prior to the induction of diarrhoea from castor oil. The ethanolic extract were given to mice in 250 mg/kg, 500 mg/kg and 750 mg/kg dose and the standard drug (loperamide) in 2 mg/kg. Mice were observed for frequency and weighed the stool.

Results: The qualitative phytochemical screenings were performed to assess the presence of flavonoids, tannins, saponin, phenol, phytosterol and diterpene. The anti-microbial activity showed at 45% w/v concentration against *S. aureus*, *Klebsiella*, *P. aeruginosa* and *S. typhi* except for *E. coli*. The anti-diarrhoeal activity was 85.71%, 88.14% and 90.43% inhibition of defecation and 89.43%, 93.40% and 95.88% inhibition of weight of stool output for ethanolic extract and for loperamide was 66.71% and 76.23% for % inhibition of defecation and % inhibition of weight of stool output.

Conclusion: The result showed that the phytochemicals present in *Psidium guajava* contains flavonoids, tannins, saponin, phenol, phytosterol and diterpene. The anti-microbial activity of P. *guajava* is effective against *S. aureus*, *Klebsiella*, *P. aeruginosa* and *S. typhi* except for *E. coli*. The anti-diarrhoeal activity of ethanolic extract of P. *guajava* is positive at 250 mg/kg, 500 mg/kg and 750 mg/kg dose on swiss albino mice.

Keywords: Aamba, *Psidium guajava*, *S. aureus*, *E. coli*, Norfloxacin, tannins.

INTRODUCTION

Natural products were used as the therapeutic agents in different diseases are present from ancient time. Use of herbal drugs is found in worldwide with different methods and for different purposes. The herbal medicine goes back to the time as the civilization itself. The remnant scrolls of papyrus Ebers, a sixteenth century old scroll, consists of 800 formulae and 700 different drugs. In China, the oldest known use of herbs is around 3000 BC and it contained 365 drugs. Similarly, in India, Ayurveda as a text were documented in 'Charak' and 'Sushrutha' Samhita. Charak made fifty groups of ten herbs where as Sushrutha arranged 760 herbs in 7 distinct sets based on their common properties [1,2].

According to WHO, the total number of medicinal plants used for the treatment is around 20,000. The traditional therapeutic practices, that have been existing before the development of the modern

medicine, are still found to be in use. Since the traditional medicines are affordable by majority of people, WHO has been promoting this traditional medical system in developing countries. According to the WHO the 8% of the people in Africa uses traditional methods for treatment.

Nepal has a climatic difference due to variation of altitude from 68 m to 8848 m. The Phyto-geographer have divided Nepal into six bioclimatic forest vegetation types. Tropical forests account for 1829 species of flowering plants and 81 species of pteridophytes. Subtropical have 1400 species of flowering plants and about 177 endemic species. Nepal represents 2.2% of global plants of which are 245 are endemic. Out of which 10% are medicinal.

Diarrhoea

Diarrhoea is a condition characterized by discharge of semi-solid or watery fecal matter from the bowel three or more times in a day [3]. It consists of increased bowel movements, fluidity, frequency of wet stool and accompanied by increased secretion and decrease absorption of fluid, losing electrolyte and water. (Fontaine). The common reason for causing diarrhoea is gastrointestinal infection from viruses, bacteria and parasites. The cause on non-infectious diarrhoea is related to side effects of drugs, toxins (food poisoning), chronic diseases or, anti-biotics. Besides other pathological conditions, usually four major mechanisms are responsible for pathophysiology in electrolyte and water transportation, such as increasing of luminal osmolarity and electrolyte secretion, decreasing of electrolyte absorption, and acceleration of intestinal motility ultimately decreasing of transition time [4].

Diarrhoea is considered to be the major cause of morbidity and mortality for the billions of rural populations and it is the second largest killer among under five children in Nepal [5]. Generally, the treatment of diarrhoea is non-specific, and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements [4]. For the treatment of diarrhea, medicinal plants are a potential source of anti-diarrheal drugs [6,7]. Moreover, many international organizations including WHO have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices. Many herbs and plants extract are used in the treatment of diarrhoeal disease, Guava (*Psidium guajava*) leaf is one of them.

According to WHO, diarrhoea is second leading reason of death of children under five years of age.

WHO has introduced a program for diarrhoeal control, which involves the use of traditional herbal medicines. Oral rehydration therapy in diarrhoeal control fails in high stool output. Symptomatic therapy with anti-motility agents is contraindicated in infectious diarrhoea. The advances for the treatment of diarrhoea with probiotic is still under development so, use of medicinal plants may aid in cost effective approach (Figure 1).

Figure 1: *Psidium guajava* leaves and flowers.

Classification:

Kingdom	: *Plantae*
Subkingdom	: *Tracheobionata*
Super-division	: *Supermatophyta*
Division	: *Magnoliophyta*
Class	: *Magnoliopsida*
Sub-Class	: *Rosidae*
Order	: Myrtales
Family	: *Myrtaceae*
Genus	: *Psidium L.*
Species	: *Guajava*
Vernacular name	: Aamba, Belauti (Nepali), Amrood (Hindi), Guava (English)

Morphology:

Colour	:	Green
Odour	:	Aromatic
Taste	:	Bitter
Size	:	5-15 cm long × 3-7 cm broad
Shape	:	Leaf blade is elliptic to oblong in shape

Guava (*Psidium guajava* Linn) is a small tropical tree that grows up to 35 feet tall; it is widely grown for its fruit in tropics. It is a member of the *Myrtaceae* family, with about 133 genera and more than 3,800 species. The leaves and bark of *Guajava* tree have a long history of medicinal uses that are still employed today. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids.

Guava leaves show many pharmacological activities like anti-inflammatory, anti-microbial, anti-diarrhoeal, anti-oxidant, anti-diabetic, anti-spasmodic, anti-cough, hepatoprotective. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have demonstrated anti-bacterial activity. Quercetin is thought to contribute to the anti-diarrheal effect of guava, it is able to relax intestinal smooth muscle and inhibit bowel contractions activity [8].

OBJECTIVES

General objective

• To evaluate the anti-diarrhoeal and anti-microbial activity of *Psidium guajava* leaves.

Specific objectives

• To evaluate the anti-diarrhoeal activity of ethanolic extract of *Psidium guajava*.

• To evaluate the anti-microbial activity of ethanolic, methanolic and water extract of *Psidium guajava*.

• To determine the phytochemical screening of ethanolic, methanolic and water extract of *Psidium guajava*.

LITERATURE REVIEW

Usage of the modern medical services seems unreachable in very rural areas hence use of plants as a medicine is still on practice in Nepal. Since, natural source for the treatment is less expensive and available in the surroundings, government and WHO has been encouraging people to use traditional methods. The *Guajava* leaves have been used for the treatment of the diarrhoea in rural areas and our research is to find the benefit of that plant in such disease. The literature that we choose for our research was taken mostly from the other researches and some from the books.

The research project of our, entitled 'Evaluation of anti-diarrhoeal and anti-microbial activity of the *Psidium guajava* leaves' was done to study the properties of the leaves in preventing diarrhoea and microbials. The other objective was to find the phytochemical properties of P. *Guajava* leaves.

Phytochemical properties of the P. *Guajava* according to the various literature like Mortan , Bijauliya et al. and Baby J. have found that it contains tannin, triterpenoids (crategolics, guaijavolic, oleanolics and ursolic acids) and essential oils containing ß-sitosterol, ß-bisabolene, ß-cariophyllene, aromadendrene, ß-salinene, guaijaverine, nerolidiol, flavonoid: quercetin, pentacyclic triterpenoid, guajanoic acid, saponins, carotenoids, lectins, leucocyanidin, ellagic acid, amritoside, beta-sitosterol, uvaol, oleanolic acid and ursolic acid [8-10].

According to the Bijauliya Rk and et.al. found on their research that the Gajava leaves contains properties like antibacterial, anti-diarrhoeal, antihyperglycemic, anti-malarial, anti-inflammatory, anti-cancer, antioxidant activity etc. [8].

The research done by the Chulasiri M. et.al., Jaiarj , Abdelrahim and Goncalves described that the aqueous extract of guava leaves were effective against microbial strains like *Aeromonas hydrophila*, *Shigella spp*. and *Vibrio spp*., *Staphylococcus aureus* and *β-Streptococcus group A*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and in addition, anti-rotavirus activity has also been reported to exist in these extracts [5,11-13].

In the phytochemical composition research done by Baby J., found that the main constituent in the guava leaves are the flavonoid called Quercetin. He thought that the anti-diarrhoeal property is due to presence of Quercetin and it causes the reduction in motility of intestine [8].

Borah et.al. investigated on the modification of anti-diarrhoeal activity of ethanolic extract of P. *Guajava* Linn. leaves with respect to change of season, namely, autumn and spring. Phytochemical scrutiny disclosed that small fluctuation of phytoconstituent content was observed. The extract was initially assayed for its possessions in castor oil induced diarrhoea at different doses (200, 400 and 600 mg/kg p.o). There was statistical noteworthy lessing ($p<0.05$) in the number of wet feces by 78.33% during spring season and 78.26% during autumn season at 600 mg/kg body weight and when compared to negative control rats, which might be due to change in climate or due to alteration in plant biosynthesis pathway in different season [14].

The research entitled 'Contents and antibacterial activity of flavonoids extracted from the leaves of *Psidium Guajava*' by Rattanachaikunsopon and Phumkhachorn, found that the dried leaves of the P. *Guajava* contains more flavonoids than the fresh leaves. But the certain flavonoids had a less concentration than that of the fresh leaves extract like morin-3-O-lyxoside, morin-3-O-arabinoside, Quercetin-3-O-arabinoside and Quercetin. His study showed that the concentration of quercetin in fresh and the dried leaves were 63.9 and 179.3 respectively. In his study for the anti-bacterial property of the flavonoid (Quercetin) against several strains of spoilage and foodborne pathogenic bacteria including *B. stearothermophilus, B. thermosphacta, E. coli* O157:H7, *L. monocytogenes, P. fluorescens, S. enterica, S. aureus* and *V. cholerae* showed different degree of inhibition in all the bacteria [15].

In the research by Gricilda et.al. the anti-diarrhoeal activity was observed using 'castor oil induced diarrhoea' mentioned in the other research by Awouters et al. with the modification. The mice were fasted for overnight before the experiment. Three groups of experiment got the doses of 3, 7.5 and 15 mg. They received 0.3 m of castor oil orally after 30 min and the mice were observed for 4 h. During that time the first diarrhoeic feces, total number of fecal output as well as number of feces excreted by animals in 4 h were noted with weight. We followed this methodology for our research [16,17].

MATERIALS AND METHODS

Collection and processing of plant materials

We collected the *Psidium Guajava* leaves from the compound of the Valley College of Technical Sciences, Sitapaila, Kathmandu. The leaves were collected by hand picking method, we collected about 10 kg of *Psidium Guajava* leaves. The leaves were then cleaned by tap water to clean the dust. The plant was identified by the National Herbarium and Botanical Laboratory, Godawari, Lalitpur.

Identification of the plant constituents

Different test was carried out for the study of chemical constituents present in this plant. The alkaloid test, Glycosides test, Flavonoid test, Tanin test, Carbohydrates test, Saponin tests were carried out.

Extraction

The leaf samples were washed in tap water, dried in the shade for 2 weeks, and placed into a blender to be grounded into powder. Three solvents will be arranged in increasing polarity; methanol (99%), ethanol (96%), and boiling distilled water were used for the maceration extraction procedure. The mixtures will be made by dissolving 50 gm of powder with 750 ml solvent in a sterile beaker wrapped in aluminum foil to avoid evaporation and exposure to light for 4 days at room temperature. The flasks will be stirred periodically. After 4 days of soaking in solvent, the mixtures will be transferred to 50 ml tubes and centrifuged for 10 min at 4,000 rpm at room temperature. The supernatant was collected and it was dried using rotary evaporator at 45°C and 40 rpm. The yield, a dark green residue (~6-7 gm) was obtained from the 50 gm of powder for every solvent [11].

Phytochemical screening

The phytochemical screening of methanol, ethanol and distilled water extract was done to identify the main chemical constituents present in P. *Guajava* leaves. The following tests were carried out.

• Detection of alkaloid

Wagner's test: 2 ml of HCl was added in 5 ml sample extract and then 1 ml of Wagner's reagent was added.

• Detection of glycosides

Legal's test: Extracts were treated with Sodium nitroprusside in pyridine and sodium hydroxide.

• Detection of flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution.

• Detection of tannins

Gelatin test: To the extract, 1% Gelatin solution containing sodium chloride was added.

• Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of Carbohydrates.

Molisch's test: Filtrates were treated with 2 drops of alcoholic α-Naphthol solution in a test tube.

• Detection of saponin

Foam test: 0.5 gm of extract was shaken with 2 ml of water.

• Detection of phenols

Ferric chloride test: Extracts were treated with 3-4 drops of Ferric Chloride solution.

• Detection of phytosterols

Salkowski's test: Extracts were treated with Chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand.

• Detection of diterpenes

Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops Copper Acetate solution [18].

Anti-microbial test

The anti-microbial activity was done by using plate diffusion method.

Media

The media used in this study were Nutrient Agar, Mac-Conkey agar, Blood agar, Mannitol-Salt agar, Nutrient broth, Triple Sugar Iron (TSI), Muller-Hinton Agar, *Salmonella-Shigella* agar, Peptone water and Glucose Phosphate broth.

Test organism

Bacterial strains of *Staphylococcus aureus*, Salmonella typhi, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* were obtained from National Public Health Laboratory Biosafety, Teku. The micro-organisms were inoculated in the broth and kept in the refrigerator until use.

Isolation of micro-organism

The obtained micro-organisms were isolated in a selective media and incubated in 37ºC for 24-48 hours. Incubated micro-organisms were then confirmed by using Gram-Staining test and viewed under microscope for its morphology.

Identification test for the organism

• Indole test: Inoculate the tryptophan broth with broth culture of the test organism in tryptophan broth. Incubated at 37°C for 24-48

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hours in ambient air. Then added 0.5 ml of Kovac's reagent to the broth culture [19].

• **TSI test:** The TSI (Triple Sugar Iron Agar) was prepared in a sterilized test tube and left to cool in a slanted position. With a sterilized straight inoculation needle touch the top of a well-isolated colony. Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant. Opening of test tube was covered with cotton and loosely covered by aluminum foil then incubated the tube at 35°C in ambient air for 18 to 24 hours [20].

• **Methyl red test:** MR-VP broth is used for both MR Test and VP test. Only the addition of reagent differs, and both tests are carried out consecutively. Two tubes containing MR-VP Broth with a pure culture were inoculated. The broth is left in incubation for 4 days in 35 ºC. A few drops of methyl red is added to the test tube [21].

• **Voges-proskauer test:** The micro-organisms were incubated in MR/VP broth for 24 hours at 35ºC. Then the 0.6 ml of 5% α-Naphthol followed by 0.2 ml potassium hydroxide of 40% was added in this order. The tube was then shaken and slightly exposed to the oxygen and kept for standing for 15 min [22].

• **Catalase test:** Small number of colonies of micro-organism was transferred to the glass slide with the help of sterile loop or the swab. A drop of 3% H_2O_2 was added and mixed [23].

• **Citrate test:** The Simmons-Citrate agar was prepared in a slanted position. Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old. Incubated for 24 hours at 35 ºC [24].

Anti-microbial activity

The media for the anti-microbial activity was prepared, when the temperature of the media is around 40 ºC, it was poured onto the petri-plates and cooled down aseptically. The micro-organism (1*10^8 cfu/ml) was spread onto the media and let it dry aseptically. The sterile borer was used to create the well and different concentration of extract and the standard anti-biotics were poured. We used two standard drug Norfloxacin and Amoxicillin. The plate is then let to incubate for 24–48 hours at 35 ºC. After incubation the zone of inhibition is recorded using Vernier caliper. The reference standards were obtained from the Central Laboratory, Teku, Nepal [25].

Anti-diarrhoeal activity

Experimental animals: The animals used for our project work were Swiss Albino Mice (20-40 gm). They were housed in the Valley College of Technical Sciences, Sitapaila, Kathmandu, Nepal. They were kept in a maintained container with light (12-hour cycle) and allowed to free of food and water.

Method: To determine anti-diarrhoeal activity of ethanolic extract of *Psidium guajava* leaves (EEPGL), COID (Castor Oil Induced Diarrhoea) model was conducted by following described method. 30 mice were randomly divided into five equal groups (n=5) namely control group, standard group and three treated groups. Before the day of experiment all the mice were fasted for 12 hours. The day of experiment control group received only distilled water 2 ml per mice while standard group received loperamide 2 mg/kg as standard and three treated groups received at the dose of 750 mg/kg, 500 mg/kg and 250 mg/kg body weight, respectively. Mice were housed in separate cages with paper placed below for collection of fecal matters. Firstly, extract and drug were given orally to treated groups and standard group respectively. In control group, only distilled water was given orally. Then, one hour later castor oil (1 ml per mice) was used to induce diarrhoea in all mice. The number of both hard and soft pellets will be counted at every hour [16,17].

RESULTS

Phytochemical screening

The result of the phytochemical screening from the leaves of P. *Guajava* shows the presence of different phytochemicals prepared in methanolic, ethanolic and distilled water extract. The qualitative assessment of different phytochemicals detect during investigation were presented below (Table 1).

S.N.	Test	Ethanolic extract	Methanolic extract	Distilled water extract
1	Alkaloid (Wagner's Test)	-	-	-
2	Glycoside (Legal's Test)	-	-	-
3	Flavonoid (Lead acetate Test)	+	+	+
4	Tanins (Gelatin Test)	+	+	+
5	Carbohydrate (Molisch's Test)	-	-	-
6	Saponin (Foam test)	+	+	+
7	Phenol test (Ferric chloride test)	+	+	+
8	Phytosterol	+	+	+

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	(Salkowski's test)			
9	Diterpene (Copper acetate test)	+	+	+

Table 1: Result of phytochemical screening.

Anti-microbial property

The 45% methanolic, ethanolic and distilled water extract of P. *Guajava* showed significant activity against *S. typhi*, *Pseudomonas aeruginosa*, *S. aureus* and *Klebsiella*. Any concentration of the extract didn't show any activity against *E. coli*. It has greater activity against *S. typhi*, *P. aeruginosa*, *S. aureus* and *Klebsiella* (Figure 2 and Tables 2 and 3).

Drug	**Zone of inhibition in mm**				
	S. aureus	***S. typhi***	***Klebsiella***	***P. aeruginosa***	***E. coli***
Norfloxcin	30	37	33	35	34
Amoxicillin	28	25	26	0	24
Dist. Water (45%)	22	24	20	30	0
Ethanol (45%)	25	25	24	26	0
Methanol (45%)	24	20	18	24	0

Table 2: Result of anti-microbial activity of P. *guajava*.

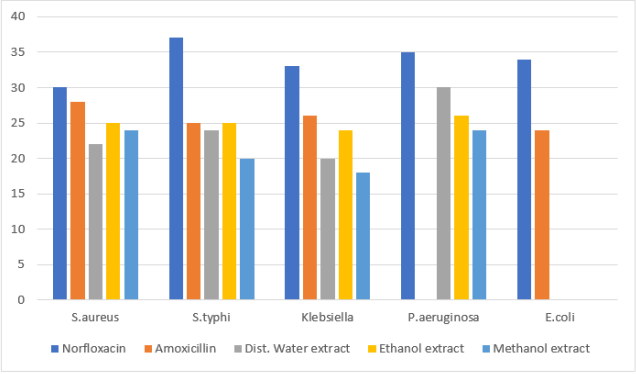

Figure 2: Bar diagram showing anti-bacterial activity of *P. guajava* extract against standard.

S.N.	**Test**	***E. Coli***	***P. aeruginosa***	***Klebsiella***	***S. aureus***	***S. typhi***
1	Indole test	+	-	-	-	-
2	TSI test	+	+	+	+	+
3	Methyl red test	+	-	+	+	+
4	V.P test	-	-	+	-	-
5	Catalase test	-	-	-	+	-
6	Citrate test	-	+	+	-	-

Table 3: Result of Identification test for micro-organism.

Anti-diarrhoeal property

The ethanolic extract of P. *guajava* showed significant activity in castor oil induced diarrhoea. The higher the concentration the better the anti-diarrhoeal activity showed the extract of *guajava* (Table 4 and Figures 3-6).

Groups	**Dose**	**Total no. of feces**	**Total weight of the feces**	**% Inhibition of the defecation**	**% Inhibition of Wt. of Stool Output**
Control	0 mg/kg	7.00 ± 1.317	.820283 ± 0.123	0	0
	250 mg/kg	1.00 ± 0.516	0.08667 ± 0.0554	85.71*	89.43*
Guava extract	500 mg/kg	0.83 ± 0.543	0.05417 ± 0.0346	88.14*	93.40*
	750 mg/kg	0.67 ± 0.333	0.03383 ± 0.0283	90.43*	95.88*
Loperamide	2 mg/kg	2.33 ± 0.558	0.1950 ± 0.07557	66.71*	76.23*

a. Values are represented as mean ± S.D. (n=6)

b. *P<0.001, when compared with control group (One-Way ANOVA test)

Table 4: Effect of ethanolic extract of *P. guajava* leaves on diarrheic mice against standard.

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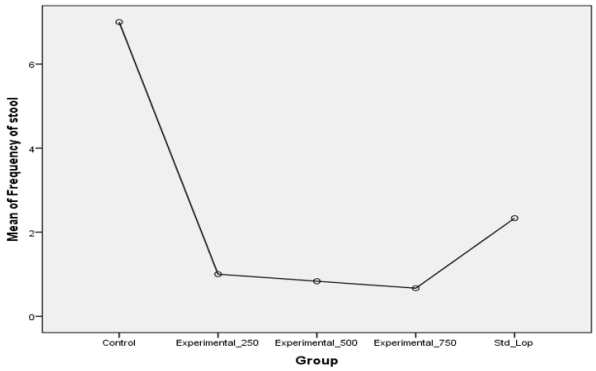

Figure 3: Mean of Frequency of stool.

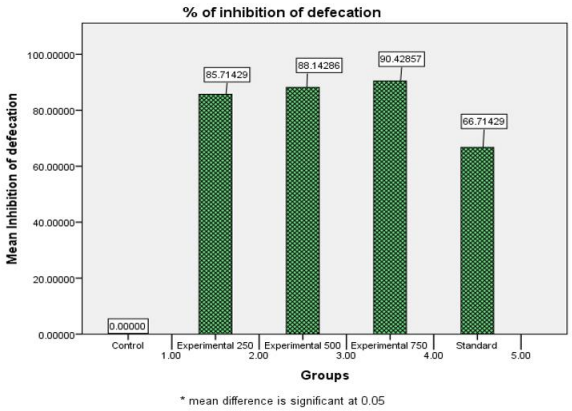

Figure 4: % of Inhibition of defecation.

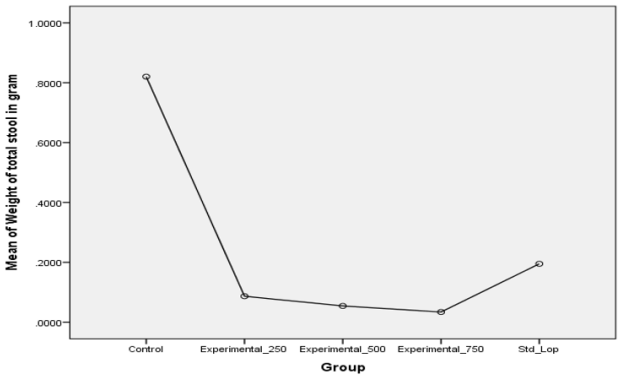

Figure 5: Mean of weight of total stool in gm.

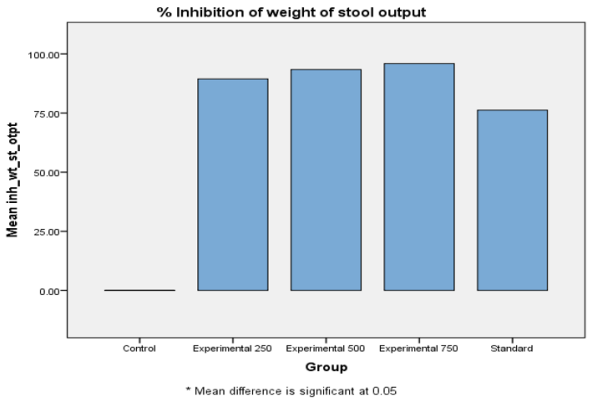

Figure 6: % Inhibition of weight of output.

DISCUSSION

It is known that Castor oil cause changes in the intestinal mucosal membranes for water and electrolytes, resulting in decrease of water and electrolyte [26]. The active component of the oil is Ricinoleic acid which produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins [27]. Because of these reason Castor oil results in a hyper-secretory response (decreasing Na^+ and K^+ absorption), simulating peristaltic activity and diarrhoea [28].

The result of ethanolic extract of P. *guajava* showed dose dependently the anti-diarrhoeal activity in the castor oil induced diarrhoea in the mice. The extract decreased the frequency of purging and inhibited the severity of diarrhoea. Although the reason for the anti-diarrhoeal activity of the P. *guajava* leaves extract could not be established in this study but other study has shown that anti-diarrhoeal activity is due to the Quercetin which inhibits the AcetylCholine release in G.I. tract [29]. Quercetin being a prominent constituent in P. *Guajava* leaves showed to have spasmogenic effects of various agonists (Carbachol, Potassium Chloride etc.) on guinea-pig isolated ileum [30].

The study of the anti-microbial activity of the P. *guajava* leaves extract showed that it is effective against the bacteria like *P. aeruginosa*, Salmonella typhi, *S. aureus* and *Klebsiella* at 45% concentration but didn't showed any anti-microbial activity against *E. coli*. Although one study showed that the organism similar to *E. coli* called C. rodentium is prevented from causing further diarrhoea [31]. It is shown that the lectin found in the *guajava* leaves were bound with *E. coli*, preventing its adhesion to the intestinal wall thus preventing infection [32].

The phytochemical study of the P. *guajava* showed the presence of the constituent like tannin, flavonoid, saponin, tri-terpinoids and di-terpinoids. The main constituent in the leaf extract of the P. *guajava* is found to be Quercetin, a flavonoid, which is the reason for the anti-diarrhoeal and anti-microbial activity [8].

CONCLUSION

The study was focused on anti-diarrhoeal, anti-microbial activities and phytochemical screening of *Psidium guajava* found in the Sitapaila, Kathandu. The presence of flavonoids, tanins, saponin, triterpinoids and diterpinoids. The presence of these phytochemicals showed that it is source for medicinal values.

The anti-diarrhoeal activity was shown by the ethanolic extract in mice model which provides evidence of its use as treatment for diarrhoea in traditional medicine. The presence of different phytochemical constituent gave the leaf extract the medicinal properties. It also showed anti-microbial activity at 45% concentration against micro-organisms like *S. aureus*, *S. typhi*, *Klebsiella* and *P. aeruginosa*. This result encourages for the development of the anti-microbial drugs from plants at minimum side effects.

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