e-ISSN:2321-6182
p-ISSN:2347-2332

Research & Reviews: Journal of Pharmacognosy and Phytochemistry

Comparative Study of Tannin Fraction from *Psidium guajava* Linn. Leaves and Bark for Wound Healing Activity

Chamakuri Subba Rao*

Department of Pharmacognosy, DR. C.S.N Institute of Pharmacy, Andhra University, Bhimavaram, India

Research Article

Received date: 05/09/2015
Accepted date: 02/11/2015
Published date: 12/11/2015

***For Correspondence**

Chamakuri Subba Rao, Department Of Pharmacognosy, DR. C.S.N Institute of Pharmacy, Andhra University, Bhimavaram, India.

E-mail: majindar@mopipi.ub.bw

Keywords: *Psidium guajava*; Tannin Fraction; Wound healing activity

ABSTRACT

The Ethnobotanical studies and folklore claiming reviewed that the leaves and bark of the plant *Psidium guajava* Linn. are used for wound healing activity. From the literatures we found that poly-phenolic compounds like tannin have significant free radical scavenging activity in bacterial associated diseases.

Based on the above information of *Psidium guajava* is selected for this Research work to separate the tannin fraction from the *Psidium guajava* Linn. leaves and bark. For study the anti-bacterial activity of tannin fraction of *Psidium guajava* separated from leaf and bark.

INTRODUCTION

The Medicinal plants are of awesome worth in the field of treatment and cure of many diseases. Throughout the years, investigative exploration has extended our insight into the compound impact and arrangement of the dynamic constituents which focus the restorative properties of plants [1-3].

India is an ancient heritage of traditional medicine. Materia medica of India provides a lot of information on the folklore practices and traditional medicine. Based on various indigenous system including Ayurveda, Siddha and Unani.

In western medicine continues to show the influence of ancient practices. More recently, there has been interest in other products from traditional systems of medicine artemisinin is an active anti-malarial compounds isolated from *Artemisia annua*, a constituent of the Chinese anti-malarial preparation qinghaous and forskolin was isolated from Coleus forskohlii, a species used in ayurvedic preparations for cardiac disorders. A new standardized preparation, artemether has recently been introduced for treatment of drug resistant malaria, and new analogues of Forskolin are being tested for a variety of uses [4].

Nature has provided an excellent storehouse of remedies to cure all the ailments of mankind. In ancient days, almost all the medicine used was from natural sources, particularly from plants and plants continue to be an important source of new drugs even now. The importance of biological, chemical and pharmacological evaluation of plants derived agents used in the treatment of human ailments has been increasingly recognized in the last decades [5].

In the next of our series on so much Japanese plant we glance at guava or dicot genus *guajava* Linn [6,7]. In people medication, extracts of roots, bark and leaves ar accustomed treat stomach flu, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, cough, raw throat, inflamed gums, and variety of different conditions. This plant appeared warrant associate comprehensive review

Guava which is scientifically known as *Psidium guajava* Linn is well known plant to all of us, the plant have many medicinal values, we are extract the medicinal values from roots, leaves and barks etc.. Guava fruits are preferred for diabetic patients and

e-ISSN:2321-6182
p-ISSN:2347-2332

used to treat several diseases like gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, cough, sore throat, inflamed gums. It is the commonly available plant in all over India, [8-12]

PHYTOCHEMICAL STUDIES

Collection aqnd Authentication

The plant specimen (Leaves and bark) for the proposed study was collected during the month of July 2010 from the garden of Vels university, Pallavaram, Chennai. It was identified and authenticated by Dr. P. Jayaraman, Director of Plant Anatomy research center (PARC), Tambaram, Chennai. A voucher specimen No. PARC /2010/594 has been deposited for further reference.

Extraction

The leaves and bark of *Psidium guajava* Linn. were shade dried and coarsely powdered. About 300 gm of powdered drug was extracted with ethanol by cold maceration method after 72 hrs of maceration it was filtered. After complete extraction the extraction was concentrated by distilling off the solvent and then evaporated to dryness under reduced pressure using vaccum flash evaporator. Then it was extracted successively with solvents of increasing polarity such as petroleum ether, chloroform, ethanol and aqueous it's to yield its fraction. All the fractions were evaporated under vaccum its color and consistencies were observed.Percentage yield was calculated on the air dried basis [13-18]. The results were tabulated in **Tables 1-3.**

Table 1. The percentage yield of *Psidium guajava* leaves.

Tannin leaf fraction (TLF)	Percentage Yield (%w/w)	Colour	Consistency
Ethanol extract	9.8	Blackish Brown	Thick semi solid
Hexane fraction	0.90	Pale yellow	Greasy
Chloroform fraction	3.42	Green	Semisolid
Aqueous fraction	2.52	Brown	Greasy

Table 2. The percentage yield of *Psidium guajava* bark.

Tannin bark fraction	Percentage Yield (%w/w)	Colour	Consistency
Ethanol extract	10.3	Blackish Brown	Thick semi solid
Hexane fraction	0.88	Pale yellow	Greasy
Chloroform fraction	4.42	Green	Semisolid
Aqueous fraction	3.52	Brown	Greasy

Table 3. Isolation of tannin fraction.

Extract/Fraction	Percentage Yield (%w/w)	Color	Consistency
Tannin leaf fraction	8.8	Light brown	Greasy
Tannin bark fraction	9.6	Brownish	Greasy

Phytochemical Screening

The leaf and bark tannin fractions were subjected to qualitative phytochemical test for identification of constituents. The results shown in **Tables 4-6.**

Table 4. Phytochemical screening of *Psidium guajava* leaf

Chemical Test	Petroleum ether Fraction	Chloroform Fraction	Aqueous Fraction	Ethanol Fraction
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	-
Glycosides	-	-	-	-
Flavonoid	-	-	+	+
Tannin	-	-	+	+
Terpenoids	-	+	-	+
Oil and fats	-	-	-	-
Steroids	+	+	-	+
(-) indicates absent; (+) indicates present				

Table 5. Phytochemical Screening of *Psidium guajava* bark.

Chemical Test	Petroleum ether fraction	Chloroform Fraction	Aqueous fraction	Ethanol fraction
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	-
Glycosides	-	-	-	-
Proteins	-	-	-	-

e-ISSN:2321-6182
p-ISSN:2347-2332

Amino acids	-	-	-	-
Saponins	+	-	-	+
Flavonoids	-	-	+	+
Tannin	-	-	+	+
Terpenoids	-	+	-	-
Oil and fats	-	-	-	-
Steroids	+	+	-	+
(+) indicates present; (-) indicates absent				

Table 6. Phytochemical screening of *Psidium guajava* leaf and bark (dried powder).

Chemical Test	Dried powder (Leaf)	Dried powder (Bark)
Alkaloids	-	-
Protein	-	-
Amino acids	-	-
Saponins	+	-
Flavonoids	+	+
Tannin	+	+
Terpenoids	-	-
Oil and fats	-	-

PHARMACOLOGICAL STUDIES

Wound healing activity [19-25]

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. A number of traditions came to dominate the practice of herbal medicines for various effective human benefits at the end of the twentieth century. With a view to increasing the wide spectrum of medicinals usages, the present day requires a new biologically active fraction which exhibit wound healing activity as local applications. The availability of market products capable of stimulating the process of wound repair is still limited. Hence the present investigation was focused in the direction of establishment of tannin fraction in th form of ointment for wound healing activity.

Formulation of ointment [26-28]

Ointment

Ointment is soft semisolid preparation meant for external application to the skin or mucous membrane. They usually contain a medicament dissolves, suspended or emulsified in the base.

Preparation of ointment procedure

Cetostearyl alcohol, white soft paraffin and yellow soft paraffin were melted together. Wool fat was dissolved separately in purified water and warmed it to almost same temperature (about 60^{o}C). Add the warmed 5% of tannin leaf fraction (TLF) and 5% tannin bark fraction (TBF) separately to the melted mixture and stir thoroughly. Allowed to cool, and packed in suitable container

Excision wound model [29-32]

Under light ether anesthesia an impression of 500 sq mm was made on the shaved back of the rat as described in Morton and Malone. The skin of the impressed area was excised carefully. Animals are kept in separate cages. The day on which wound was made consider as day'0' (Zero). Animals divided into four groups of each with 5 animals. Group A considers as control and treated with simple ointment (eg. Bees wax, Cetosteryl alcohol etc.), group B consider as standard and treated with 5% w/w Povidine iodine ointment, group C and group D are *psidium guajava* treated group and applied ointment 5% respectively. The percentage of wound closure was recorded on day 4,8,12 and 16. Wound area was traced and measured plan metrically with the help of sq mm graph paper. Number of days required for falling of the eschar without any residual raw wound gave the period of epithelization **(Tables 7 and 8).**

Table 7. Thin layer chromatography of leaf and bark fraction of *Psidium guajava* Linn.

S. NO	Test extract	Solvent system	Detecting agent	Number of spots	R_f value
1	Standard (Gallic acid)	Toluene: acetone :Glacial acetic acid(3:1:2)	5% $Fecl_3$	2	0.91
2	TLF	Toluene: acetone :Glacial acetic acid (3:1:2)	5% $Fecl_3$	1	0.91

e-ISSN:2321-6182
p-ISSN:2347-2332

3	TBF	Toluene: acetone :Glacial acetic acid (3:1:2)	5% $Fecl_3$	1	0.89
R_f: Retardation factor, TLF: Tannin Leaf Fraction, TBF: Tannin Bark Fraction					

Table 8. Effect of tannin leaf fraction and tannin bark fraction (ointment) of *Psidium guajava* on excision model.

Group	% Wound contraction on				Epithelization time (days)
	4thday	8th day	12th day	16th day	
A Control(simple ointment base)	15.78 ± 3.01	31.24 ± 2.50	44.89 ± 3.22	60.78 ± 4.22	23.4 ± 0.66
B Standard Povidone Iodine.5% w/w	34.50 ± 2.65***	58.94 ± 2.98***	87.20 ± 3.98***	97.77 ± 4.20	17 ± 0.58***
C TLF 5% w/w	27.36 ± 2.15*	47.56 ± 3.55**	58.94 ± 4.50***	78.67 ± 4.82***	19.2 ± 0.55*
D TBF 5% w/w	32.72 ± 3.45***	58.45 ± 2.92***	87.25 ± 2.95***	94.81 ± 2.89***	17 ± 0.81***

The values are expressed as Mean ± SEM, n=5 in each group. If *P<0.05, **P<0.01 and ***P<0.001 vs. control
TLF: Tannin Leaf Fraction, TBF: Tannin Bark Fraction.

RESULTS

The present work covers study on anti-inflammatory and wound healing activity of the leaves and bark of *Psidium guajava* Linn.

Extraction

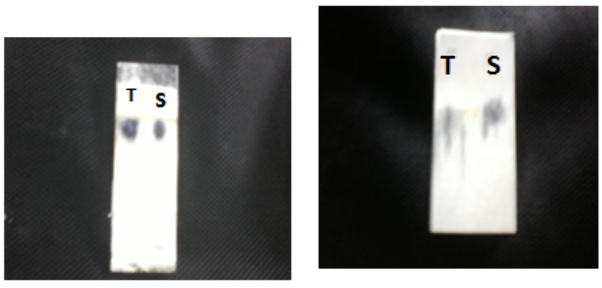
The successive extraction leaves of *Psidium guajava* Linn. was done in the order of increasing polarity i.e. Petroleum ether, Chloroform, ethanol and aqueous **(Tables 1-3).**

Phyto-chemical screening

The phytochemical test was carried out for the identification of various constituents. It answers positively for saponins, flavonoids, Phenolic compounds, tannin, terpenoids and steroids **(Tables 4-6).**

Chromatography (TLC) of extract

The extracted compound identified compare with standard sample (Gallic acid) by TLC. It was performed using solvent system only glacial acetic acid (10 ml) with 5% ferric chloride ($fecl_3$) as detecting agent. The result observed in the R_F values of standard (0.93 and 0.87), tannin rich fraction R_f value (0.91&0.85) The results were reported in **Table 7** and **Figure 1**



T: Tannin Leaf Fraction (TLF) T: Tannin Bark Fraction (TBF)
S: Standard.

Figure 1. TLC of TLF and TBF of *Psidium guajava* Linn.

Wound Healing Activity

Effect of combined extract on excision wound model

In excision wound model, the results indicates the topical application of combined plant extract formulation in different concentration (5% and 5%) have demonstrated significant reduction in the wound area. The 5% ointment of combined plant extract treated animals showed faster epithelialization of wound (17 ± 0.81) than the animals treated with 5% combined extract ointment (19.2 ± 0.55). The period of epithelialization was (17 ± 0.58) in the case of standard drug 5% w/w standard Povidone Iodine Ointment treated animals **(Figure 2).**

e-ISSN:2321-6182
p-ISSN:2347-2332

Figure 2. Before wound healing rat and after wound healing rat.

DISCUSSION

The Ethnobotanical studies and folklore claiming reviewed that the leaves and bark of the plant *Psidium guajava* L., are used for wound healing, anti-inflammatory and antibacterial activities. The young leaves are used as tonic in the diseases of the digestive function and is said to be remedy for toothache. Tannin has a broad scale of biological activities among which anti-inflammatory and wound healing effects stands out. *Psidium guajava* Linn. Belongs to Myrtaceae, is a wide spread plant in India and commonly used as for antiseptic, anthelmintic, wound healing and in inflammatory conditions. It has a high content of tannin substances reviewed from literature.so the present work was focused to isolate tannin rich fraction and it was evaluated for anti-inflammatory and antibacterial activities. The tannin rich fraction of leaf and bark of the plant was formulated in the ointment form and studied for wound healing activity.

Phytochemical study

Phytochemical screening was carried out to identify the phyto constituents present in the ethanolic extracts and its fraction. Phytochemical screening of tannin isolation shows the presence of tannin.

The total tannin content was estimated by using spectrophotometer method. The results indicate that the content of tannin was found to higher in the plant. TLC was done for the tannin fraction on support of the chemical test since it showed blue color spot with 5% ferric chloride, confirmed the presence of tannin. It was identified as gallic acid by comparing its R_f value with that of standard gallic acid.

Wound healing activity

Wound healing, a complex sequence of events, is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by wounding of tissues. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue. The results of present study indicates that 70% hydro alcoholic leaf and bark extracts ointment of title plants at both strengths (5% leaf and 5% bark) exhibited significant wound healing promoting activity. However, this effect was found to be concentration related fashion where5% ointment promotes significant wound-healing activity by increasing cellular proliferation, formation of granulation tissue, synthesis of collagen and by increase in the rate of wound contraction as compared to the control animals. This was evident by faster rate of wound closure and epithelization period in excision wound model. Further phytochemical studies are needed to isolate and characterize the individual tannin, and identify the compounds .which is responsible for anti-inflammatory and wound healing activity.

CONCLUSION

From this study, it is concluded that *Psidium guajava* Linn. Leaf and bark tannin fraction have significant wound healing models. That tannin rich fraction of leaf and bark of the plant was formulated in the ointment form and it was screened for In vivo wound healing. It showed significant percentage wound protection at the tested concentration.

The wound healing activity is probably due to the presence of tannin (gallic acid). Further studies need to be isolate individual tannin and explore its biological potency by various preclinical and clinical trials of the isolated compounds.

REFERENCES

1. Abdelrahim S.I., Atragboul A.Z., Omer M.E.A. Screening of *Psidium guajava* aqueous bark and methanolic extract and antibacterial activity. Fitoterpia. 2002; 73: 713-715.

2. Adeyemi. Phytochemical and trypanocidal activity of Ethanolic leaf Extract in rats infected with trypnosoma brucei brucei. Journal of medicinal plants Research. 2009; 3(5): 420-423.

3. Agarwal S.S, Paridavi M. Herbal drug Technology. Nirli Prakashan Publications, 2007; 1.

4. Ajai Kumar, Tandon S, Naquvi AA, Kahol AP. Composition of leaf and twigs oil of *Psidium guajava*. Indian Perfumer. 2005; 49(1): 73-75

5. Chaterjee A, Chandra S, Pakrashi. 'The treatise on Indian Medicinal Plants'. National Institute of science communication and information resource, New Delhi. 2003; 4: 14-16.

6. Kumar A, Lakshman K, Jayaveera KN. Estimation of Gallic Acid, Rutin and Quercetin in Terminalia chebula by HPTLC. Jordan Journal of Pharmaceutical Sciences. 2010; 3(1)

7. Amarawiez R, Traszynska A, Pegg RB. Antioxidative and radical scavenging effect of phenolics from Vicia sativum, Fitoherapi. 2008; 79: 121-122.

8. Chanchal KR, Jagadeesh V, Kamath. The hepato protective activity of *Psidium guajava* Linn. Indian Journal of Expermental Biology. 2006; 44: 305-311.

9. Dhara AK, Gebremariam B, Nag C. Neuropharmacological evolution of Psidium gujava fruit .Indian Journal of pharmaceutical sciences. 1994; 56 (4): 164.

10. Deguchi Y, Miyazaki K. Anti hyper glycemic and anti-hyperlipidimic effects of guava .Phytopharm. 2010; 11(03): 26

11. Fatima S, Jadhav MJ. Studies on brown rot of guava: isolation, pathogenicity and control. Bioinfolet. 2006; 3(1): 74.

12. Fu HZ, Luo YM, Li Cj. Isolated psidials. Phytopharm. 2010; 11(02): 31.

13. Fathilah AR, Yasmin R, Thman O, Zubaidah. The effect of Piper beetle and Psidium gujava extracts. Journal of Oral sciences. 2006; 48: 71-75.

14. Gandhisan R, Thamaraichelvan AB. Anti-inflammatory action of Lannea coromandelica Hrbc membrane stabilization, Fitotherapia. 1999; 62: 82-83.

15. Gorman, Zheng and Wang. Antioxidant and antiproliferative activities of methanolic extracts of Perilla frutescens. Journal of Medicinal Plants Research. 2010; 4(6): 477-483.

16. Gupta VK, Misra AK, Gaur RK, Current status of fusarium wilt disease of guava. Journal of Biotechnology. 2010; 9(2): 176-195.

17. Harborne JB. Phytochemical method guide to modern technique of plant analysis. Springer, 1998; 83.

18. Joshi SG. The Indian Medicinal Plants. Oxford and IBH Publishers, 2003; 293.

19. Jimenez A, Rincon M, Pulido R, Saura-Calixto. Guava fruits as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry. 2001; 49(11): 5489-5493.

20. Jairaj P, Ongkrajang Y, Thongapraditchote S. Effect of Psidium gujava leaf extract on the bleeding time. Phytotherapy Research. 2000; 14(5): 388-391.

21. Ji XD, Pu QL, Garraffo M. Characterization of the compounds present in the essential oil. Journal of essential oil research. 1991; 3(3): 187-189.

22. Kaudil FE, El-Sayed, Michael NH. Flavonoid composition of the leaves of *Psidium guajava*. Asian journal of chemistry. 1997; 9(4): 871-872.

23. Khare CP. 'The Indian Medicinal Plants' An illustrated plants Dictionary. 2007; 522.

24. Kiritikar, Basu. The Indian Medicinal Plants'. International Book Distributors Publishers, Dehardun, 1999; 2: 1046.

25. Kalyon R, Shivakumar H. Wound Healing Potential of Leaf Extracts of Ficus Religiosa on Wistar albino strain rats. International Journal of Pharm Tech Research. 2009; 1(3): 506-508.

26. Luna S, Sandoval E, Lozoya. Anatomical characteristics of matured guava leaf and Quality control of the herbal raw material. Fitoterpia. 1986; 9: 223-229.

27. Lal G, Sen NL. Effect of nitrogen, zinc and manganese fertilization on soil composition and yield of guava. Hamdard medicus. 2003; 46(3): 82-85.

28. Lutterodt A, Ismail RH. Inhibition of microlax-induced experimental diarrhoea with narcotic like extracts of Psidium gujava. Journal of Ethnopharmacology. 1992; 37(2): 151-157.

29. Matsuzaki K, Ishii R, Kobiyama. Structures of new benzophenone and quercetin galloyl glycosides. Phytopharm. 2010; 12:.41.

30. Mcculum. Cereal chemistry, proanthocyanins from grape seeds. 1989.

31. Mercadante A, Steck A, Pfander. Carotenoids from the flesh of Brazilian red guavas. Journal of Agricultural and food chemistry. 1999; 47(1): 145-151.

32. Meckes M, Calzada F, Tortoriello. Depressant activity of isolated terpenoids from *Psidium guajava*. Phytotherapy Research. 1996; 10(7): 600-603.