Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com (ISSN : 2277 –4572)
Research Article

PHYTOCHEMICAL SCREENING AND ANALGESIC EFFECT OF ETHANOL LEAF EXTRACT OF *Psidium guajava* (Linn)

Wazis CH [1]*, Timothy SY [1], Yesufu HB [2], Mashi JS [3], Saminu D [1]
[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria
[3]Department of Pharmaceutical Services, Federal Medical Centre Gombe, Nigeria
*Corresponding Author Email: chamawazis@yahoo.com

DOI: 10.7897/2277-4572.04667

Received on: 19/11/15 Revised on: 07/12/15 Accepted on: 14/12/15

ABSTRACT

Psidium guajava is an important food crop and medicinal plant available in tropical countries, widely used in food and folk medicines around the world. The aim of this study is to investigate the analgesic property of ethanol leaf extract of *Psidium guajava.* The *Psidium guajava* L. leaves were freshly collected from cultivated source on the in December 2014 at the Agricultural Science Farm of University of Maiduguri. This was dried, powdered and macerated with 95% ethanol to give the ethanol extract. The extract was then used to evaluate the preliminary phytochemistry and analgesic property. The preliminary phytochemical screening revealed the presence of alkaloids, steroids and triterpenoids, carbohydrate, cardiac glycosides, tannins and flavonoids. The leaf extract of *Psidium guajava* exhibited significant analgesic effect in acetic acid and tail immersion induced pain at the dose of 250 to 1000 mg/kg in rats. The ethanol leaf extract of *Psidium guajava* contain some active phytochemical constituents which may be responsible for the observed analgesic activity and this amply justify the traditional use of this plant as a pain reliever.

Keywords: *Psidium guajava,* phytochemistry, analgesic effect

INTRODUCTION

Human beings have always pondered and tried to understand why they feel pain and how to reduce it. In the past, pain and disease were thought to be consequences of human wrong doing[1]. The Western concept of pain has evolved with understanding of the world around it and attitudes toward pain have changed and developed in accordance with the science and religious climate of the period[2]. The 19th and 20th century saw the advent of new anatomical, physiological and biochemical insight and modern pain theories were developed. Modern analgesic drugs were synthesized along with new invasive procedures for pain management strategies. The older traditional beliefs, concepts and attitudes however, have not been replaced completely and have survived to some degree in modern patients to this day[3]. An analgesic or painkiller is any member of the group of drugs used to achieve analgesia (relief from pain). Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which reversibly eliminate sensation and include paracetamol (not technically analgesic), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and oxycodone.

Plants form the main ingredient of medicines in traditional systems of healing and have been the source of raw materials for major pharmaceutical drugs or companies. Roughly about 50,000 species of higher plants (about one in six of all the species) have been used medically. This represents by far the biggest of natural world in term of number of species[4]. Research on medicinal plants especially indigenous knowledge has become of great importance in present day drug development studies. This is because herbal remedies have been widely used for centuries for the effectiveness, accessibility, availability, and affordability. *Psidium guajava* L. (Family Myrtaceae) is a small tropical tree that grows up to 35 feet tall; it is widely grown for its fruits in the tropics. It is a genus of about 133 genera and more than 3,800 species. The leaves and bark of *P. guajava* tree have a long history of medicinal uses, which is still employed today[5]. *Psidium guajava* is well known traditional medicinal plant used in various indigenous systems of medicines and widely distributed throughout India[6]. In Nigeria, south Africa, Ghana and Kenya, Panama, Cuba, Costa Rica, Mexico, Nicaragua, Venezuela Mozambique, Guatemala and Argentina, guava is used traditionally for treatment of various ailments like, diarrhoea, wounds, rheumatism, lung problem, ulcer, malaria, cough, fever, inflammatory conditions, as well as diabetes mellitus[7]. It has also been reported to contain a number of phytochemicals and pharmacological active principles such as flavonoids, guayolic acid, guavanoic acid, guajadial and guajarin[8].

MATERIALS AND METHODS

Source of Plant Materials, Collection and Identification

The *Psidium guajava* L. leaves were freshly collected from cultivated source in December 2014 at the Agric Farm of University of Maiduguri and were identified by Professor Sanusi O. Sanusi (Professor of Botany) of the Department of Biological Science, University of Maiduguri.

Ethanol Extraction (Maceration Method)

The leaves collected were dried at room temperature for one week and was powdered using wooden pestle and mortar to a fine particles for ease of extraction process of active compounds.

About 500 g of the powder was weighed and then defatted using 150 ml of petroleum ether. The mixture was stirred and kept for 24 hours. It was then filtered at which 400 ml of 95% ethanol was added and kept for another 24hours before filtration. This procedure was repeated three times and the combined filtrate was subjected to rotary evaporator to obtain the dried extract of the leaf of *Psidium guajava*. The percentage yield was then calculated using dried weight of the extract and the dried weight of the starting powder material. Freshly prepared stocks were used which were prepared using water for injection Bp for the concentration of both standards and extracts just prior to the experiment.

Preliminary phytochemistry of ethanol leaf extract of *Psidium guajava*

The preliminary phytochemical analysis of the leaf extract was carried out according to standard method of Trease and Evans[9].

Acute toxicity study of ethanol leaf extract of *Psidium guajava*

The acute toxicity study was carried out based on the modified Lork's method[10]. The rats were grouped into four groups of three rats each and were administered the extract at the dose levels of 10 mg/kg, 100 mg/kg, 1000 mg/kg and 2000 mg/kg intraperitonealy respectively. This was observed for 24 hours for the sign of toxicity and death.

Acetic acid induced Writhing test

The antinociceptive activity of the ethanol leaf extract was studied using acetic acid induced writhing model in rat[11]. The animals were divided into five groups (5) with six rats in each group. The animals of test groups received test samples at the doses of 250, 500 and 1000 mg/kg body weight. Positive control group received standard drug pentazocine at the dose of 30 mg/kg body weight and vehicle control group was treated with distilled water at the dose of 2 ml/kg body weight. Test samples, pentazocine and vehicle were administered intraperitoneally 60 minutes before intraperitoneal administration of 1% acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 30 minutes[12].

Tail immersion test in rat

Albino rats weighing between 120-170 g were used for the evaluation of analgesic activity. The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in to the water bath of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 second units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of the test and standard substance. The cut off time is 15 sec. A solution of pentazocine (30 mg/kg) was prepared in normal saline water. Albino rat of either sex were divided into four different groups each containing five rats and the animals were marked individually. The animals were weighed and numbered appropriately. The test and standard drugs were given intraperitonealy. After 30 minutes, observations were made and recorded at the time interval of 30, 60 and 90 minutes[13].

Table 1: Phytochemical constituents of ethanol leaf extract of *Psidium guajava*

Phyto constituents	Method Used	Results
Alkaloid	Meyer's test	-
	Dragendorff's test	+
Saponins	Frothing test	-
	Haemolysis test	-
Steroids and triterpenoids	Lieberman's test	+
Cardiac glycosides	Salkowski test	+
Tannins	Soluble	+
	Condensed	+
Flavonoids	Lead Sub-acetate test	+
Carbohydrate	Ferric chloride test	+
	Molish's test	+
	Fehling's test	+

Table 2: Acute toxicity study of ethanol leaf extract of *Psidium guajava*

Phases	Dose (mg/kg)	Observations
I	10	-
	100	-
	1000	-
	2000	-
II		
LD_{50}	**>2000**	

- = no death

Table 3: Effect of ethanol leaf extract of *Psidium guajava* in acetic acid induced pain

Treatment	Dose (mg/kg)	Number of writhing's	% Protection
Distilled water (ml/kg)	2	46.67±0.71	
Pentazocine	30	3.83±0.31	91.79
PG Extract	1000	6.67±0.80*	85.71
PG Extract	500	9.83±1.33*	78.94
PG Extract	250	21.00±1.79*	55

PG = *Psidium guajava*, Distilled water was administered in ml/kg, n = 6. *= significant to both controls (P< 0.05)

Table 4: Effect of ethanol leaf extract of *Psidium guajava* in tail immersion induced pain

Treatment	Dose (mg/kg)	Time (seconds)		
		30 mins	60 mins	90 mins
Distilled water	2	2.40±0.24	2.80±0.37	2.40±0.24
Pentazocine	30	38.60±2.11	191.80±15.40	336.00±30.59
PG Extract	1000	12.60±0.98*	16.80±1.46*	22.00±0.71*
PG Extract	500	6.40±0.75*	13.20±1.07*	17.80±0.66*
PG Extract	250	5.40±0.40*	6.60±0.51*	8.20±0.86*

PG = *Psidium guajava*, Distilled water was administered in ml/kg, n=5, T-test, *= significant both positive and negative control (p< 0.05)

RESULTS ANALYSIS

Phytochemical constituents of ethanol leaf extract of *Psidium guajava*

The results of phytochemical screening of the ethanol leaf extract of *Psidium guajava* revealed the presence of the following phytochemical constituents; alkaloids, steroids, triterpenoid, cardiac glycosides, tannins, flavonoids and carbohydrates. However, saponin was not detected in the extract (Table 4.1).

Acute toxicity study of ethanol leaf extract of *Psidium guajava*

The results of acute toxicity study carried out on the ethanol leaf extract of *Psidium guajava* revealed the relative less toxicity. The lethal dose that can kill 50% of the rats (LD_{50}) was found to be greater than 2000 mg/kg (Table 4.2).

Effect of ethanol leaf extract of *Psidium guajava* in acetic acid induced pain

The effect of ethanol leaf extract of *Psidium guajava* in acetic acid induced pain showed a dose dependent activity in pain reduction. The activity of the extract in reducing pain in albino rats was found to be statistical significantly higher than distilled water used as negative control ($p<0.05$). However, the activity of pentazocine was found to reduce the acetic acid induced pain better than the extract at the tested doses ($p<0.05$) (Table 4.3).

Effect of ethanol leaf extract of *Psidium guajava* in tail immersion induced pain

The effect of ethanol leaf extract of *Psidium guajava* in tail immersion induced pain showed a dose dependent activity in reaction time. The activity of the extract in prolonging reaction time in albino rats was found to be statistical significantly higher than distilled water used as negative control ($p<0.05$). However, the activity of pentazocine was found to prolong the reaction time in tail immersion assessment of nociceptive activity in albino rats significantly higher than the extract at the tested doses ($p<0.05$) (Table 4.4).

DISCUSSION

The phytochemical compounds detected in the ethanol leaf extract of *Psidium guajava* L. in this present study do agree with the report of several related studies[8, 14-17] in which similar phytochemicals were detected. However, saponin detected by Shruthi *et al*[14] and Cho *et al*[18] was not detected in this present study. This may be attributable to the changes in the solvent system used. The saponin was detected in hydroalcoholic, methanol, acetone and distilled water extracts[14, 18]. Significant number of bioactive metabolites reported by several studies[16-17, 19-23] which was not detected by the present study is due to the inability of the present study to perform the screening tests due to financial constraint.

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics[24]. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P at the nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response[25].
The method has also been associated with prostanoids in general that is, increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products[26]. The significant reduction in pain threshold produced by tests and standard in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems[27]. The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain. In the present study, administration of the extract led to a significant reduction in the induced pain by acetic acid. The observed analgesic activities of *P. guajava* may be due mainly to quercetin, flavonoid and steroids contained in this plant[28]. Previous reports demonstrated that the leaves of *Psidium guajava* and some medicinal plant like *Cassia alata* are rich in flavonoids, in particular, quercetin which may be responsible for their therapeutic activity[29-30].

CONCLUSION

The plant extract contain diverse phytochemical constituents that may be responsible for the observed analgesic activity evidenced by increased reaction time in tail immersion and decreased numbers of writhing in acetic acid induced pain. This further gives credence and pharmacological basis for its traditional use for managing pain and other conditions.

REFERENCES

1. Perl ER. Ideas about pain; a historical view. Nat Rev Neurosci 2007; 8:71-80.
2. Jaros JA. The concept of pain. Critical care Nurse clinic North America 1991; 3:1-10.
3. Sabatowski R, Galvez R, chevry D, Jacquot F, Vincent E, Maisonobe P *et al*., 2004. Pre- gabalin reduces pain and improves sleep and mood disturbances in patients with post herpic neuralgia; Results of randomized, placebo-controlled clinical trial. Pain 109(1-2): 26-35.

4. Idu M. The plant called medicine; Department of plant biology and biotechnology 104th Inaugural lecture; university of Benin press 2009; 32, 57-58.
5. Nwinyi OC, Chinedu NS, Ajani OO. Evaluation of antibacterial activity of *Psidium guajava* and *Gongronema latifolium*. J Med Plants Res 2008; 2(8): 189-192.
6. Mital K, Sumitra C. Phytochemical and Pharmacognostic Evaluation of leaves of Psidium guajava L. Pharmacognosy Journal 2011; 3(23): 41-45.
7. Van Wyk, Wink M. Medicinal plant of the world, 1st edition. Briza publication pectoria South Africa 2004: 258.
8. Sanda KA , Grema HA, Geidam YA, Bukar-kolo YM. (2011)Pharmacological aspects of *Psidium guajava*. Journal of pharmacology 2011; 7: 316-342.
9. Trease GE, Evans WC. Pharmacognosy. 15th Ed London: Saunders Publishers.2002: 42-332.
10. Lorke D. A new approach to practical acute toxicity testing. Arch toxicology 1983; 54: 257-287.
11. Ahmed F, Selim MST, Das AK, Choudhuri MSK. Antiinflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie 2004; 59: 329- 333.
12. Meera S, Kumar NS, Guptatyam VSSS. Screening of anti-arthritic, anti- inflammatory and analgesic activity of a polyherbal formulation. International Journal of Pharmaceutics 2008; 4: 398-402.
13. Olaleye SB, Farombi EO, Adewoye EA, Owoyele BV, Onasanwo SA, Elegbe RA. Analgesic and anti-inflammatory effects of Kolaviron (a garcinia kola seed extract). Afr J Biomed Res 2000; 3: 171.
14. Shruthi D, Adhikari RS, Sharma T, Sajjekhan S. *A* review on the medicinal plant psidium guajava linn. Journal of Drug Delivery & Therapeutics 2013; 3(2): 162-168.
15. El-Mahmood MA. The use of *Psidium guajava* Linn in treating wound, skin and soft tissue infections, Scientific Research and Essay 2009; 4(6): 605-611.
16. Ojewole JAO. Anti-inflammatory and analgesic effects of *Psidium guajava* L. (Myrtaceae) leaf aqueous extract in rats and mice. Methods Find Exp Clin Pharmacol 2006; 28: 441-446.
17. Nundkumar N, Ojewole JA. Studies on the antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu folk medicine. Methods Find Exp Clin Pharmacol 2002; 24: 397-401.
18. Cho EJ, Yokozanawa T, Yokozawa DY, Kim SC, Shibahara N, Park JC. Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-piccrylhydrazyl radical. Phytomed 2003; 10: 544-551.
19. Kamath JU, Rahul N, Ashok KCK, Lakshm SM. *Psidium guajava*. In a review. Int Green Pharm 2008: 9-24.
20. Akinpelu DA, Onakoya TM. Antimicrobial avtivities of medicinal plants used in folkloric remedies in south-western Nigeria. Afr J Biotechnol 2006; 5(11): 1078-1081.
21. Begum S, Hassan SI, Ali SN, Siddiqui BS. Chemical constituents from the leaves of *Psidium guajava,* Nat Prod Res 2004; 18(2): 135-140.
22. Dio A, Satri NV. Purification and characterization of polygalacturonase inhibitory proteins from *Psidium guajava* Linn. (Guava) fruit. Plant science. 2003; 164,147-56.
23. Wilson CW, Shaw PE. Terpene hydrocarbons from *Psidium guajava,* Phytochemistry. 1978; 17: 1435-6.
24. Gené RM, Segura L, Adzet T. *Heterothecainuloides* anti-inflammatory and analgesic effects. J Ethnopharmacol 1989; 60: 157-161.
25. Bentley GA, Newton SH, Star J. Studies on the Anti-nocicrptive action of agonist drugs and their interaction with opiod mechanisms. Br J pharmacol 1983; 79: 125-128.
26. Bentley R, Jongney S, Delevalcee F. Release of Prostaglandin E and F in an analgesic Reaction and its Inhibition. Eur J Pharmacol. 1980; 51: 17-22
27. Bensreti MM, Sewell RD. Selective effects of dopaminergic modifiers on antinociception produced by different opioid receptor agonists. Pro Br Pharmacol Soc 1983: 70.
28. Morales MA, Tortorielo J, Mekes M, Paz D, Lozoya X. Calcium antagonist effect of quercetin and its relation with the spasmolytic properties of Psidium guajava L. Arch Med Res 1994; 25: 17-21.
29. Lozoya X, MeckesM, Abou-Zaid M, Tortoriello J, Nozzolillo C and Arnson JT. Quercetin glycosides in *Psidium guajava* L. leaves and determination of a spasmolytic principle. Archive of Medical Research 1994; 25: 11-15.
30. Timothy SY, Lamu FW, Rhoda AS, Adati RG, Maspalma ID, Askira M. Acute toxicity, phytochemistry and antibacterial activity of aqueous and ethanolic leaf extracts of *Cassia alata* linn. International Research Journal of Pharmacy 2012; 3(6): 73-76.

How to cite this article:
Wazis CH, Timothy SY, Yesufu HB, Mashi JS, Saminu D. Phytochemical screening and analgesic effect of ethanol leaf extract of *Psidium guajava* (Linn). J Pharm Sci Innov. 2015;4(6):304-307 http://dx.doi.org/10.7897/ 2277-4572.04667

Source of support: Nil, Conflict of interest: None Declared