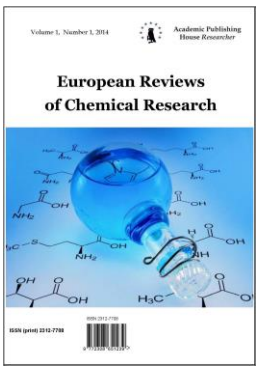

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Evaluation of the Effects of *Psidiumguajava* Leave Extracts on Biochemical Indices of two Liver Enzymes and Some Haematological Parameters in Rabbits

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Abstract

The influence of *Psidiumguajava* leaf extract on the biochemical indices of the liver function and some haematological parameters in rabbits was assessed. The plant leaves were collected from the College of Agriculture, Bauchi garden and authenticated by two agronomist; S. Adamu and A. Bununu of the department of Agricultural Technology, college of Agriculture, Bauchi. The powdered leaves of *P. guajava* was extracted with ethanol and distilled water using maceration for 2 days. Phytochemical screening of the resulting ethanol and aqueous extracts was carried out using standard procedures. Eight rabbits were randomly grouped into four (4) groups T_1, T_2, T_3 and T_4. Rabbits in T_1 (control) were administered orally with distilled water after acclimatization period. Groups T_2, T_3 and T_4 received 200mg/kg of the ethanol leaf extract of *P. guajava* for a period of 40 days.

Preliminary phytochemical screening of the ethanol extract revealed the presence of saponins, alkaloids, flavonoids, tannins, steroids, terpenes and phenols which varies in the aqueous extract. The liver function test revealed that the serum ALT and AST were found to be within the normal range of 10-45U/L and 10-120U/L respectively. However, there is a significant increase in the red cells production and the hemoglobin concentration.

The results of the study suggested that the ethanol extract of *Psidiumguajava*leaves extract exhibit the hematopoietic potential and has no negative impact on the liver and may be hepatoprotective and hence, the findings may be of clinical importance considering the various reported medicinal values of the plant.

Keywords: *Psidiumguajava*, Hepatic functions, ALT, AST and Phytochemical screening.

1. Introduction

It is generally known that the consumption of a variety of local herbs and vegetables by man contribute significantly to the improvement of human health, in terms of curing and or prevention of diseases. Plants have long served as a useful and natural sources of the therapeutic agents (Gupta et al., 2014).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants is attributed to the chemical substance that produces a definite

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physiological action on the human body. Many of these medicinal plants are used as spices and food plants (Abbasi et al., 2015).

Moreover, traditional medicine is greatly relied upon especially by rural dwellers for the treatment of various ailments. Traditional doctors are the dispensers of such concoctions (Belonwu et al., 2013; Ferngren, 2014). Guava (*P. guajava*) is a common shade tree or shrub indoor-yard gardens in the tropics. The tree is easily identified by its distinctive feature as thin, smooth copper-coloured bark that flakes off showing the greenish layer beneath (Hiwale, 2015).

It has been reported that physiochemical analysis of *P. guajava* leaf products revealed the presence of more than 20 isolated compounds, including alkaloids, antholyanins, and arytenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triteerpens and vitamin C (80 mg per 100kg of *P. guajava*). The main active constituent in the plant is reported to be quercetin. Spasmolytic and antidiarrheal effect are report to be associated with its quercein derived, flaronoids and glycoside, which support use of this ancient leaf remedy in treating gastrointestinal disorders. The decoction made from the leaves and or bark of *P. guajava* has been reported to be, used by many tribes for diarrhea, destiny sore throats, vomiting, stomach upset and to regulate menstrual periods throughout the tropical Amazon and India (Hiwale, 2015). Moreover, tender leaves are also reported to be chewed for bleeding guns and bad breath and it is said to prevent hangovers if chewed before drinking. According to the report, Indians throughout the Amazon gargle a leaf decoction for mouth sore, bleeding gums or use lit as douche for vaginal discharge and to tighten vaginal wall after childbirth. The medicinal importance of the plant have been attributed to their photochemical content. Thus phytochemical analysis of the plants is predicated by the need for drug alternatives of plant origin, made imperative or essential by the high cost of synthetic drugs for example, *L. owariensi* leaves have been report to contain various secondary metabolites of medicinal value including saponins, tannins, alkalolids and flavonoids. These secondary plant metabolites extractable by various solvent exhibits varied biochemical and pharmacological actions in animals when ingested. Within the recent decades a good number of medicine plant have been reported be employed in folk medicine in the treatment of anaemia (Nath, Jain, 2015).

Anaemia is one of the clinical conditions that constitute a serious health problem in many tropical countries as result of the prevalence of different forms of parasitic infections, including malaria. Anaemia condition is characterized by a decrease in the level of circulating hemoglobin less than 13g/dI in males and 12g/dI in females. In the tropics, due to the endemicity of malaria and other parasitic infection, between 10 to 20 % of the population is reported to possess less than 10g/dI of Hb in the blood. The determination of the hematological indices provides physiological information on a proper assessment. According to (Edagha et al., 2014) accurate determination of blood parameters remain the only sensitive and reliable foundation for ethical and rational, research, diagnosis, treatment and prevention of anaemia. The major concern of scientific community with regards to medicinal plants and hematological studies focuses on the measures that can maintain the normal hematological state of being and reverse any negative hematological status associated with various anaemic conditions (Edagha et al., 2014). This study therefore is to partly assess the hematological potential of *Psidiumguajava* leaves extract in rabbit model, considering the fact that different parts of the plant have been reported to be useful in the management various diseases. On the whole, this work aims at reporting the effect of *P. guajava* leaves extract on the biochemical indices of liver function and some hematological parameters in rabbits.

2. Materials and methods

Experimental Site

The research work was carried out at Chemistry/Biology Laboratories of the School of Science and Technology, AbubakarTatari Ali Polytechnic and Pathology Laboratory section of Darusssalam Health Clinic Center, Dutsen Tanshi Bauchi, Bauchi State, Nigeria.

Collection and Identification of Plant Material

Fresh leaves of P. *guajava* were collected from the schools garden. The identification and authentication of the plant was done by a Botanist, Ibrahim Shuaibu, College Agriculture, Bauchi. The leaves were sorted, shade dried, pulverized to powder and stored in a clean container for onward analysis.

Extraction

Extraction with ethanol

The Powdered leaves (200g) was extracted with 400ml ethanol using maceration method for 2days with occasional shaking. The extract was filtered using Whatmann No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40^{o}C) to obtain a gummy greenish product (28g) subsequently referred to as the crude ethanol extract (EE).

Extraction with distilled water

The Powdered leaves (100g) was extracted with 200ml distilled water using maceration method for 2days with occasional shaking. The extract was filtered using Whatmann No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40^{o}C) to obtain a gummy greenish product (28g) subsequently referred to as the aqueous extract (AE).

Preliminary Phytochemical Investigation

Portion of the fractions each was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins and steroids/triterpenes and alkaloids using standard procedures.

Test for Alkaloids

0.5g of the extract was stirred with 5 ml of 1 % aqueous hydrochloric acid on a water bath and filtered. 3ml of the filtrate was divided into two. To the first 1ml few drops of freshly prepared Dragendoff's reagent was added. To the second, 1 drop of Meyer's reagent was added and observed.

Test for Flavonoids and Phenols

Ferric chloride test: To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

Test for Anthraquinones

0.5g of the extract was shaken with 5ml carbon tetrachloride, this was filtered and 10 % dilute ammonia solution was added. The mixture was shaken and observed.

Test for Saponins

0.5g of the extract was shaken with distilled water in a test tube. It was allowed to stand for 10 minutes and observed.

Test for Steroids and Triterpenes

Liebermann-Buchard test: A small portion of the extract was dissolved in chloroform. Equal volume of acetic anhydride and concentrated H_2SO_4 were added down the test tube and observed.

Test for Tannins

Lead Sub-acetate Test: To a small portion of the extract, distilled water was added. 3-5 drops of lead acetate solution was added and observed.

Test for Carbohydrates

Fehling's Test: To a small portion of the extract, distilled water was added. 2ml Fehling's

Test for Glycosides

Legal'stest: To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed.

Preparation of Animal Sample

A total of 8 rabbitscontaining four young and four adult rabbits of either sex (2kg) obtained in Bauchi metropolis were used for the study. They were kept in the School Laboratory Garden for 40 days so as to acclimatize with the environment. The test animals were divided into four groups of two rabbits each (containing a mixture of one young and one adult rabbit each). T_1 serves as the control, T_2, T_3 and T_4 serving as the test groups.

Mode of Administration and Dosage

T_1 serving as the control group received distilled water (placebo) while the ethanol leaf extract of *M. indica*was administered orally to the test groups (T_2, T_3 and T_4). The dosage of administration sustained was 200ml/kg daily in divided doses for a month.

Blood Collection Procedure

The blood was collected from the central auricular artery (ear) with a 20g needle. Vasodilation was achieved with the aid of heating lamps, 70 % alcohol swabs and warm compression.

Liver Function Test (ALT & AST) using Randox Reagent

The ALT & AST test procedure was conducted according to the manufacturer's instruction in the following three steps;

Step 1:

Two test tubes were set as TEST and BLANK. 200 U/L of reagent 1 was added to both test tubes. To the TEST tube, 40 U/L of serum was added to it and 40 U/L of distilled water to the BLANK tube and incubated at 37°C in water bath for 35 minutes.

Step 2:

200 U/L of reagent 2 was added to both the TEST and BLANK tubes, incubated for 20 minutes at room temperature.

Step 3:

To the TEST and BLANK tubes, 2000 U/L of sodium hydroxide (NaOH) solution was added and incubated for 5 minutes at room temperature. The result was displayed at 530 mm with the aid of photoelectric colorimeter. ALT and AST were calculated below;

ALT (U/L) = Optical density of sample mixture × concentration = OD of sample mixture x 160

AST (U/L) = Optical density of sample mixture × concentration = OD of sample mixture x 350

PCV

Capillary tube (75mm) was filled to approximately with EDTA and ant coagulated blood (3 quarter of its length). The excess blood was wiped from the outside of the tube and scatted with a sealer. The tubes were placed in a microhaematocrit centrifuge with the sealed end pointing outwards. The inner lid was firmly secured and the outer lid was also closed and centrifuge for five minutes at 11,000 revolutions per minute. When the centrifuge stopped, the tubes were removed and read as the fraction of red cells column to the total length of the sample.

3. Results

The result of phytochemical screening, effects of Psidium*guajava* leaf extracts on rabbits serum enzymes and haematological indices are presented in Tables 1-3 respectively;

Table 1. Phytochemical Constituents of Ethanol and Aqueous Extracts of P. *guajava*

Constituents	Test	Observation	Inference	
			EE	AE
Saponins	Frothing	Frothing persist for 15mins	++	+
Flavonoids	$FeCl_2$	Green or violet ppt	++	+
Tannins	Lead subacetate	Cream ppt	+	+
Steroids & Terpenes	Lieberman-Buchard	Blue-green color at interphase	+	+
Anthraquinones	Borntragers	Pink or violet	-	-
Phlobatannins	Lead subacetate	Cream ppt	-	-
Reducing Sugar	Fehling's	Bluish black color	+	+
Glycosides	Fehling's	Red ppt	++	-

\+ = present
++ = present in high concentration
\- = absent

Table 2. Effect of the ethanol leaf extract of *P. guajava* on rabbit serum enzyme

Group	ALT (U/L)	AST (U/L)
T_1	28.80	38.50
T_2	32.80	45.50
T_3	30.40	45.50
C	25.60	31.50
Normal range	10-45	10-120

Key: T=Test; C=Control

Table 3. Effect of the ethanol leaf extract of *P. guajava* on rabbit haematological indices

Group	PCV (%)	Hb (g/dl)
T_1	48.0	15.40
T_2	44.0	14.70
T_3	49.8	14.00
C	36.0	124.00
Normal range	33-50	94-174

Key: T=Test; C=Control

4. Discussion

The result of preliminary phytochemical analysis of the ethanol (EE) leaf extract of *P. guajava*revealed the presence of all the constituent tested including flavonoids, saponins, steroids, tannins except Phlobatannins and anthraquinones. The aqueous extract (AE) revealed the presence of all the constituents except anthraquinones, Phlobatanninsand glycosides. These constituents have been reported to be responsible for most biological activities of plants (Shabbir, et al., 2013).

The results obtained for serum alanineaminotransferases (ALT) and aspartateaminotransferases (AST) were found to be within the normal range of 10-45U/L for ALT and 10-120U/L for AST. However, there was no significant alteration with the level of the serum enzymes in the control rabbit which is an indication that the ethanol leaf extract of *P. guajava*did not alter the stoichiometry of the liver marker enzymes and the liver. The packed cell volume (PCV) and hemoglobin concentration were the only heamatologicalparameters tested. According to the result obtained in the analysis, there was a slight variation between the PCV and Hb concentration in the test animal and the control. Hence, the extract has no adverse effect on the circulating red blood cell as well as the Hb concentration but rather brings about the slight increase in the production of red blood cell as well as the Hb concentration. This may be attributed to the presence of active constituent that promote red cell production in the plant extracts.

5. Conclusion

The results of the study suggested that the ethanol extract of *P. guajava*maymaybe hepatoprotective and hence, the findings may be of clinical importance considering the various reported medicinal values of the plant.

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