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Quantitative Phytochemical Evaluation, Oral and Intraperitoneal Toxicity Studies of the Crude Methanol Leaf Recipe Extract of *Carica papaya, Psidium guajava and Terminalia catappa*

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ABSTRACT

The cost and accessibility of conventional medical treatment make some northern Nigerian residents rely heavily on the use of plants for the treatment of major and minor ailments. Due to this widespread acceptance and perceived potency of plants, *Carica papaya, Psidium guajava and Terminalia catappa* are used individually and as a recipe in the management of sickle cell disease and other complications associated with the disorder. This paper evaluated crude methanol leaf extract of the recipe (*Carica papaya, Psidium guajava and Terminalia catappa*) extracted with 80% using maceration technique. The three plants were formulated into a recipe in the ratio of 1:1:1 and the yield obtained was concentrated under reduced pressure. The results of the quantitative phytochemical evaluation of crude methanol leaf recipe extract revealed the following composition (%): Alkaloid, 5.60; flavonoids, 0.54; phenols, 1.56; tannins, 5.40 and saponins, 2.80. Similarly, the result of the intraperitoneal toxicity threshold of the recipe extract was calculated to be 2,154 mg/kg bd.wt of albino rat. While the oral lethal dose (LD_{50}) was calculated to be 5000 mg/kg bd.wt of albino rat. Therefore, it is concluded that the methanol crude recipe extract of *Carica papaya, Psidium guajava and Terminalia catappa* contain appreciable percentages of bioactive metabolites of pharmacological significance and is non-toxic using both routes of administrations.

KEYWORDS: Quantitative Phytochemical evaluation, Toxicity, *Carica papaya*, *Psidium guajava*, *Terminalia catappa*, Albino Rats.

INTRODUCTION

The three medicinal plants (*Carica papaya, Psidium guajava and Terminalia catappa*) selected for this study were carefully picked after a review of their ethnomedicinal, ethnopharmacological efficacies, availability and application in the management and treatment of sickle cell disease from sources such as West African Herbal Pharmacopoeiae, Anti-sickling Herbs, An Inventory of Ethnobotanicals Used in the Management of Sickle Cell Disease in Nigeria, Useful Medicinal Plants of West Africa, Medicinal Plants of Ghana among others [1-6].

The plants belong to different families. However, they are locally formulated into a recipe to manage the crises in sickle cell, malaria fever, typhoid and in the treating infections in both males and females. According to Aravind *et al.*, [7] and Iwu, [8] *Carica papaya* is the only species in the genus *Carica*. It belongs to the plant family *Caricaceae*, while Kaneria and Chanda, [9] and Haida *et al* [10] reported that *Psidium guajava* L is native to South America and belongs to the family, *Myrtaceae*. The third plant *Terminalia catappa* according to Chen and Li, [11] belongs to the *Combretaceae* family and is known to originate from subtropical and tropical zones of India and pacific oceans.

The formulation of medicinal plants recipe has a long history among traditionalist and herbalist, with a lot of successes being attributed to the practice. This practice is widely accepted and applied by significant number of the population in Nigeria, largely due to the large deficit in the health care sector. Thus, the populace resort to plants and their recipe as the source of medication. More so, it is universally recognised that plants and synthetic pharmaceutical products can be used to managed and treat symptoms of diseases [12]. Similarly, Fulata et al. [13], reported that the recipe formulated from these three plants could be an excellent candidate for the development of plant drug for the management of sickle cell disease (SCD) because of its significant polymerisation inhibition of red blood cells, sickling reversal and inhibition effects [13].

However, no study was known to have been carried out on the toxicity and quantitative phytochemical evaluation of the methanol crude recipe of the three plants. Hence, the aim of research is to quantify the amount of some active principles in the crude methanol leaf recipe

extract of the three plants and evaluate the crude recipe's toxicity level using two routes of administration (intraperitoneally and orally) on albino rats.

MATERIALS AND METHODS

MATERIALS

Some of the major materials used are UV spectrophotometer, rotary evaporator and electronic weighing balance.

SAMPLE PREPARATION

The three plant samples (leaves) collected were dried under shade and pulverized using wooden mortar and pestle. One hundred and fifty grams (150 g) of each of the pulverized plants materials were pooled together and formulated into a recipe in (1:1:1) ratio and extracted with 80% methanol using maceration technique. The crude recipe extract was concentrated at low temperature to minimize the degradation of thermolabile components [14]. The crude recipe was evaluated for phytochemical constituents and toxicity

DETERMINATION OF PHYTOCHEMICALS

Total Tannins

The tannic acid content of the sample was determined calorimetrically by the method of Price and Butler [15]. The Absorbance was measured at 720 nm within 10mins and the concentration was calculated in mg/cm^{3}using the formula:

$$\text{Tannins (mg/cm}^3\text{)} = \frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}} * 100$$

Total Saponins

Saponin was determined according to the method of Birk *et al.* [16], as modified by Hudson and El-Difrawi [17]. The percentage saponin was calculated using the formula:

$$\%\ Saponins = \frac{W3 - W2}{W1} * 100$$

Where,

W_3 = weight of beaker and residue after evaporation to dryness

W_2 = weight of beaker alone

W_1 = weight of sample

Total Alkaloid

Alkaloid content was determined by the method of the Association of Official Analytical Chemists [18]. The percentage was calculated by the formula:

$$\%\ Alkaloids = \frac{W3 - W2}{W1} * 100$$

Where,

W_3 = weight of filter paper and alkaloids after drying

W_2 = weight of filter paper alone

W_1 = weight of sample 100 = scaling factor to convert to percentage

Total Phenolics Content

The total phenolic content of the extract was determined by adopting the method as described by Wolfe *et al.* [19].

Total Flavonoid

Flavonoid content was determined by the method of the Association of Official Analytical Chemists [18]. Percentage flavonoid was calculated using the formula:

$$\%\ Flavonoids = \frac{W3 - W2}{W1} * 100$$

Where,

W_3 = weight of beaker and flavonoids

W_2 = weight of beaker alone

W_1 = weight of sample

100 = scaling factor to convert to percentage

Acute Toxicity Study

A total of 24 rats with body weights of 100-125 g were used in this study and the analyses were carried out using the methods of Lorke's [20]. The extract was administered using two routes of administration (orally and intraperitoneally) in two phases. The animals were monitored for 24 hours and later 14 days for mortality and general behaviour changes. The medium lethal dose (LD_{50}) was calculated as given below:

$$LD_{50} = \sqrt{(a^2 + b^2)}.$$

Where a = least dose that kills a rat, while, b= highest dose that does not kill any rat.

RESULTS AND DISCUSSION

The result of the quantitative phytochemical evaluation of crude methanol leaf recipe extract revealed the following composition (%) of metabolites: Alkaloid, 5.60; flavonoids, 0.54; phenols, 1.56; tannins, 5.40 and saponins, 2.80 as presented in Table 1.

Table 1: Quantitative Phytochemical Contents of Crude Methanol Leaf Recipe Extract

Phytochemical Constituents (%)	Quantity (Mean±SEM)
Alkaloids	5.60
Flavonoids	0.54
Phenol	1.56
Tannins (mg/cm^3)	5.40
Saponins	2.80

The result of the intraperitoneal (ip) administration toxicity evaluation of the methanol crude recipe extract revealed that the toxicity level of the extract was examined in two phases; at lower dose (first phase) and higher dose (second phase). The intraperitoneal toxicity study revealed that Intraperitoneal *(ip)* administration resulted in the mortality of an animal in the group that was administered 2,900 mg/kg in the second phase and its lethal dose was estimated to be 2154 mg/kg. Hence, the crude methanol recipe extract is considered safe as presented in Table 2.

Table 2: Median Lethal Dose (LD_{50}) of Crude Methanol Leaf Recipe Extract (*ip*)

Phase I

Group	Dose (mg/kg)	Number of Rats	Clinical Sign	Mortality
A	10	3	None	0/3
B	100	3	None	0/3
C	1000	3	None	0/3

Phase II

Group	Dose (mg/kg)	Number of Rats	Clinical Sign	Mortality
D	1600	1	None	0/1
E	2900	1	Weakness	1/1
F	5000	1	Weakness	1/1

Intraperitoneal $LD_{50} = \sqrt{(a*b)} \Rightarrow \sqrt{(1600 * 2900)} = \sqrt{4640000}$

$LD_{50} = 2154$ mg/kg

The result of the oral (po) administration toxicity evaluation of the methanol crude recipe extract revealed that the toxicity level of the extract was examined at lower dose (first phase) and higher dose (second phase). The oral toxicity revealed that oral administration of the crude recipe extract did not result in mortality of animals in both phases of the study. Hence, the oral administration of the crude recipe extract is considered to be safe as presented in Table 3.

Table 3: Median Lethal Dose (LD_{50}) of Crude Methanol Leaf Recipe Extract (*po*)

Phase I

Group	Dose (mg/kg)	Number of Rats	Clinical Sign	Mortality
A	10	3	None	0/3
B	100	3	None	0/3
C	1000	3	None	0/3

Phase II

Group	Dose (mg/kg)	Number of Rats	Clinical Sign	Mortality
D	1600	1	None	0/1
E	2900	1	None	0/1
F	5000	1	None	0/1

No mortality was recorded up to the dose of 5000 mg/kg

The result of the quantitative evaluation of the crude methanol leaf extract of the recipe (*Carica papaya, Psidum guajava* and *Terminalia catappa*) revealed the following percentages of metabolites; alkaloids, 5.60, flavonoids, 0.54; phenols1.56; tannins, 5.40 and saponins, 2.80 (Table 1). The presence of these metabolites was earlier established by Fulata *et al.* [13] in their work on phytochemical, anti-sickling and polymerization inhibition studies of the crude methanol leaf recipe extracts of three medicinal plants. Similarly, Fulata et al., [21] in another publication attributed the anti-sickling potentials of crude methanol leaf extracts of *Psidum guajava* and *Carica papaya* to the presence of quantifiable amounts of these metabolites. Likewise, the results of this study indicate that the crude recipe extract contained appreciable quantity of bioactive compounds capable of eliciting different pharmacological responses.

Moreover, the intraperitoneal administration of the crude recipe extract was safe at the first phase Table 2, as no abnormality or mortality was observed, while in the second phase, the *ip* administration resulted in mortalities at the doses of 2900 and 5000 mg/kg. Furthermore, based on the observed responses, the lethal dose for intraperitoneally administration was calculated to be 2154 mg/kg bd.wt. According to Al-Shoyaib, et al. [22], the intraperitoneal route is the fastest route through which drugs goes into systemic circulation, hence, bypassing all interferences that may occur in the gastro-intestinal chamber. The active principles found in plants may be regarded as toxic except otherwise established [23]. Thus, the toxicity observed with the intraperitoneal administration of the crude recipe extract could be due to respiratory or central nervous system failure as was reported by Wun [24]. Hence, the manifestation of toxicity could be as a result of one or many of the constituents acting synergistically or additively to elicit their toxic effect.

Furthermore, the results of the intraperitoneal and oral LD_{50} study for the crude recipe extract presented in Tables 3 revealed that the oral administration of the crude extract was safe because it did not result in mortality at both phases of administration. However, the oral administration seems to be safe, since no abnormality or mortality was recorded up to the dose of 5000 mg/kg bd.wt. This result is in line with the works of Sekhar et al., [25]; Roy et al., [26]; Atik et al. [27], Hermione et al. [28] and Batubo [29] who reported the safety of plant extract administration on animals up to the dose 5000 mg/kg bd.wt.

CONCLUSION

From this study, despite the mortality recorded, the crude recipe extract is still categorized safe based on toxicity classification by the Organization for Economic Co-operation and Development states that LD_{50}>500 ≤ 2000 mg/kg is non-toxic. Therefore, it can be concluded that the crude recipe extract contained sufficient amount of phytochemical constituents capable of eliciting pharmacological response and is safe and practically non-toxic. However, we recommend that sub-chronic, chronic toxicities and histopathological analyses need to be carried to ascertain its effect on internal organs.

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