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Evaluation of Aqueous Leaf Extract of Phyllantus Niruri in Vitro

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Abstract

The toxicity effect of Chanca piedra (Phyllanthus niruri)leaves extract was studied on albino rats. Rats were divided into four groups, four per group. Group 1 received water and feeds only, whileonly. Groups 2, 3, and 4 were administered doses of the extract 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight respectively. Parameters studied were indices of liver and kidney function. Results showed that final body weight, serum, AST, ALP, urea, Creatinine, and some electrolytes were not affected by the administration of the different doses of the extract. ALT significantly increased by administration of 200 mg/kg and 400 mg/kg of the extract when compared with the control. Besides, there was asignificant increase in the level of K+ (P>0.05) at the dose of 200mg/kg body weight of the extract. Similarly, Na+ was also significantly increased by the administration of the extract (400 mg/kg and 800 mg/kg). These increases observed may be due to the certain phytochemicals present in the extract. The longer administration of the extract could likely not to be safe**.**

Keywords: Hepatotoxicity, kidney diseases, Phyllahntus niruri, medicinal plant, traditional medicine.

1. Introduction

Chanca piedra (Phyllanthus niruri) is a tropical plant widely spread and commonly found in wet areas of the Amazon, and it belongs to the family Euphorbiaceae[1]. The plant is a small erect, annual herb that grows 50-70 cm in height, and is quite prevalent in the Amazon and other wet rainforests [2].

Chanca piedra (Phyllanthus niruri) is a medicinal plant employed in the treatment of gall and kidney stones (active stone as a preventative), effectively utilized by the generation of Amazonia indigenous people [3]. This plant is commonly known by the Brazilians as Chanca piedra Piedrawhich also translates to “stone breaker” [4]. The plant was reportedalso has the capability to reduce pain, urinary calcium in patients with hypercalciuria, expel intestinal gas, stimulate and promote digestion, expel worms as a mild laxative, the aqueous extract was also known to inhibit HIV-1- reverse transcriptase [5, 6].Chanca piedra (Phyllanthus niruri) is used for so many purposes in the herbal medicine system. It is also employed for hypertension due to the existence of geranin, and high cholesterol levels control. Chanca piedra is used for viruses including hepatitis A, B, and C. The plant was reported to accelerate reflex maturation in neonates, and improve offspring memory while inducing no maternal or neonatal toxicity

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[7].This plant regulates kidney stones, strengthens, detoxifies, and protects the kidneys, andreduces uric acid, and increases urination, and also possessed antimicrobial attributes [8].Chanca piedra has been in top gear as an area for photochemical research since the mid-1960s where active constituentsand their pharmacological activities are determined [6]. It is one of the richest chemically sourced plants among thePhyllanthus genus having a number of flavonoids been successfully identified, including rutin and quercetin, which are well known to have significant antioxidants and chelating properties [9]. Many of the active constituents are attributed to biological active lignans, glycosides, flavonoids, alkaloids; ellagitannin and phenylpropanoids found in different parts of the plant such as the leaf, stem, and root [10] common lipids, sterol and Flavonols also occur in the plant [11]. The main phytoplant chemicals in Chanca piedra include alkaloids, saponins,Alkaloids, Saponins, ellagitannins, carboxylic acids, hypophyllanthin, methyl salicylate, lignans, ninuirins, phyllanthenol, niranthin and quercetol[11, 12].

The whole Phyllahntus niruri plant was reported to be utilized either in form of hot water extract, infusion, or in combination as a remedy for illnesses such as eye ailments, bronchitis, gastrointestinal discomfort, diabetes, kidney and liver problems, cuts and skin burns, menstrual irregularities, vaginal discharge and respiratory disease [11]. Its toxicity becomes worrisome to individuals since quantification of its concentrations is the key centre of its usage. Therefore, in higher doses,dosages, it seems to be abortive and purgative [13]. Though, considered as a pain reliever, cooling agent, as alcoholic appetiser drink, carminative as well as a digestive, diuretic, laxative, stomachic, tonic and vermifuge, constrict blood vessels, and eliminates excess uric acid [11]. In India, the boiled extract of leaves and stems were used as dyeing material or ink [14].

In recent years; there has been an increased focus on the use of alternative medicine for the treatment of many diseases [15, 16]. Most of these alternative medicines are from herbal preparations [17]. Phyllahntus niruri is one of the thousands of plants that are used traditionally to treat various ailments by people in most parts of the world [6]. However, medicinal plants produce phytochemicals as secondary metabolites during their normal metabolic processes [9]. These phytochemicals make up the pharmacologically active ingredients of the plants. The amount of these phytochemicals differvary in concentration depending on the part of the plant that is used in herbal preparation as well as the type of solvent used in the preparation of extract [18]. Some of these phytochemicals are toxic when taken in large doses [19]. Hence there is a need to evaluate the effect of taking some of these herbal products on the kidneys and liver in experimental animals before being utilized by humans. This study is designed to investigate some aspects of acute toxicity of the aqueous leaf extract of Chanca piedra (Phyllanthus niruri) in the liver and kidney of rats.

Figure 1: *Phyllantusniruri*(Source: http://herbsfromdistantlands.blogspot.com)

2. Materials and Methods

2.1 Plant Collection an The leaves of Chanca piedra (Phyllanthus niruri) were obtained at Rose Flower Garden close to Federal Government College, Maiduguri. The plant was authenticated by a plant taxonomist at the Department of Biological Science, University of Maiduguri, Maiduguri, Nigeria. The leaves were dried under shade dried and ground into a fine powder using pestle and mortar, sieved (0.8mm mesh) to obtain a fine powder sieve to make it suitable for the extraction procedure. The total weight of the sample obtained was 314g and was kept in a clean airtight container.

2.2Aqueous Extraction of Chanca piedra (Phyllanthus niruri)

From the stored powder 200 g was transferred into flask and 500ml of distilled water was added, covered with a thin foil paper, and left overnight. The solution was filtered and concentrated using a hot plate. After evaporation, a Brownish black viscous substance was obtained. Sixty-one grams (61 g) was used for further analysis.

2.3Experimental Animals

The study albino rats are sixteen (16) of both sexes and weighing between 163g and 193g. The rats were obtained from the Department of Biochemistry, animal farm unit, University of Maiduguri. Feeds and water were administered *ad libitum* they were kept at standard environmental conditions. The entire experimental procedures were carried out in accordance with the guidelines for the handling of laboratory animals.

2.4 Experimental Design.

The rats were divided randomly into four groups, I, II, III and IV, each group contain 4 replicates.The first (group I) was the normal control and II, III and IV were the test group. The rats were observed for one week before the commencement of the experiment, to acclimatize, and then taken their initial weight. The extract was orally administered daily for 28 days by intubation with groups II, III and IV receiving 200 mg/kg, 400 mg/kg and 800 mg/kg respectively. At the end of the experiment, the rats from all groups were anesthetized and put to death. The blood sample was collected and centrifuged, and the serum obtained was used for biochemical analysis.

2.5 Phytochemical Screening

Standard procedures were used to determine the bioactive constituents of Chanca piedra (Phyllanthus niruri) extracts. These procedures were carried out in the Department of Biochemistry Laboratory University of Maiduguri, the following tests were conducted: Tannins, flavonoids, and anthraquinones were determined as described by Trease and Evans [20]. Alkaloids were determined by the method of Evans [21], Saponins was determined by the method described by Kokate [22], Glycosides were determined by the method of Sofowara[23] and phlobatannin was determined as described by Ezeonu and Ejikeme, [24].

Aspartate aminotransferase and alanine aminotransferase were determined using the method of Reitman and Frankel [25], alkaline phosphatase was determined using phenolphthalein monophosphate method. Urea was determined using the diacetylmonoxime method as described by Rosenthal [26]. Creatinine was determined using Jaffe reaction method as described by Kaplan and others [27]. Sodium and potassium were determined by flame photometry, chloride was determined by the Scales and Scales method, bicarbonate was determined by back titration as described by Paussand others [28].

3.6LD_{50} Determination

LD_{50} represents the lethal dose of the aqueous extract that will kill 50% of the population and was determined by using Lorke's method [29]. The LD_{50} is calculated from the formula:

$$LD_{50} = \sqrt{D_0 X D_{100}}$$

D_0 = Highest dose with no mortality, D_{100} = Lowest dose with mortality.

3.7 Statistical Analysis

The values were presented in triplicate as mean ± SD, n = 4. Differences between the mean values were established using ANOVA. The results were considered statistically significant at $P<0.05$.

4. Results and Discussion

Table 1 shows the phytochemical constituents of Chanca piedra (Phyllanthus niruri). The present study indicates the presence of alkaloids, saponins, glycosides, flavonoids and phlobatannin in the aqueous leaf extract of Phyllahntusniruri. However, tannin and anthraquinones were absent in the aqueous extract. Many studies have also reported the phytochemical constituents present in the ethanolic and aqueous leaf extract of Phyllahntusniruri[30, 31, 32]. In addition, Kaur and others [11] reported the presence of alkaloids, anthocyanins, chlrogenic acids, coumarins, flavonoids, lignans, phenolic acids, tannins, terpenoids, and saponins. In another study Bagalkotkar and others [9] asserted that the active phytochemicals present in various parts of Phyllahntusniruri include flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins. *d Preparation*

Table 1: Phytochemical constituent of Chanca piedra (Phyllanthus niruri)

Test	Result
Tannins	-
Alkaloids	+
Saponins	+
Glycosides	+
Flavonoids	+
Anthraquinones	-
Phlobatannin	+

Key:Present: +; Absent: -

From the Table 2 result, the effect of aqueous leaf extract of Chanca piedra (Phyllanthus niruri) on weekly body weight gain in albino rats shows a slight decrease in body weights within weeks 2 and week 3. Nevertheless, it was observed to have an increase in the body weights in all the control and experimental groups after the 4th week.

Table 2: Effect of aqueous extract of Chanca piedra (Phyllanthus niruri) on weekly body weight gain in albino rats

Duration of treatment (weeks)	1	2	3	4	% Weight gain after 4 weeks
Group 1 (Control)	169.00±31.22	167.80±16.39	166.76±10.30	187.35±5.07	9.80
Group 2 (200 mg/kg)	191.50±16.26	187.60±37.64	166.50±0.72	193.25±10.80	0.90
Group 3 (400 mg/kg)	173.25±14.55	168.52±18.80	165.95±16.89	190.00±7.07	8.81
Group 4 (800 mg/kg)	169.75±15.30	164.15±10.29	163.06±4.24	187.00±5.30	9.22

Values are represented as mean ± SD, n=4

Table 3 shows the effect of aqueous extract of Chanca piedra (Phyllanthus niruri) on liver function indices in albino rats. There was no significant difference in the serum level of alkaline phosphatase (ALP) when the dosage of the extract was administered. Similarly, no significant increase in the levels of aspartate aminotransferase (AST) compared with the control.In contrast, there was a significant increase in the levels of alanine aminotransferase (ALT) between group 2 at (200 mg/kg) and group 3 at (400 mg/kg) dosage was administered when compared with the control.However, after administration of 800 mg/kg extract, there was no significant increase in the level of ALT as compared with the control group.In our current study, the results of the extract effect on liver enzymes show no toxicity as there was no significant increase in the levels of ALP and AST. This finding correlates with the results reported previously by Asare and others [33] which stateddeclared no significant difference exists after administration of single large doses of 2000 mg/kg and 5000 mg/kg aqueous extract of P. niruri. More so, Paula and others [34] also reported that there was no change in the levels of AST and ALT upon administration of 150 mg/kg to 600 mg/kg aqueous extract of P. niruri in pregnant rats .

ALT and AST are marker indicators of liver damage. The outcome of the previous and our current findings might suggest that the aqueous extract of P. niruri may not be toxic to the liver. This could be attributed to the content of antioxidants. in aqueous extract. This prediction is in line with the work of Giribabau and others, [35] who reported the in vitro antioxidant activity of aqueous extract of P. niruri in protecting the kidneys from oxidative stress by maintaining the levels of endogenous antioxidant enzymes. In addition, the work of Ezzat et aland others [36] also supports our current finding of un-toxicity of the extract. Their finding reported the in vivo administration of anaqueous extract of Phyllahntusniruri in rats (25, 50, 100, and 200 mg/kg) caused normalization of AST, ALT, and alkaline phosphatase (ALP). The study suggested that P. niruri can be used as a promising hepatoprotective agent. Its activity could be attributed to the potent antioxidant and anti-inflammatory actions of its phenolic constituents. On the contrary, this study revealed that there was a significant increase in the level of ALT after theadministration of 200 mg/kg and 400 mg/kg extract. However, there was no significant increase in this parameter when 800 mg/kg of the extract was administered. The alteration in the level of ALT as concentration increases to 800mg/kg could be that the system has adapted to the high dose.

Table 3: Effect of aqueous extract of Chanca piedra on liver function indices in albino rats

Doses (mg/kg)	ALP (iu/L)	AST (iu/L)	ALT (iu/L)
Group 1 (Control)	206.5±30.41	100.5±6.36	27±2.82
Group 2 (200)	205.5±29.79	105.5±5.12	33±1.44*
Group 3 (400)	206.9±30.53	106.5±4.94	33.5±1.94*
Group 4 (800)	207.0±31.73	100.5±6.12	29.5±2.36

The values are presented as mean ± SD, n=4. * Indicate significant difference when compared to control (p<0.05).

Table 4 shows the effect of aqueous extract of Chanca piedra (Phyllanthus niruri) on kidney function indices in albino rats. The extract administration showed no significant increase in the serum levels of urea, chloride (Cl-) and bicarbonate (HCO3-) in all the doses. The extract administration showed no significant increase in creatinine level at 400 mg/kg aqueous extract, while observed, statistically insignificant increase in the other treatment groups. In the same vein, there was a significant increase in the serum level of potassium (K+) after the administration of 200 mg/kg of the extract compared with control group. Although,

the administration of 400 mg/kg and 800 mg/kg resulted in a significant increase in sodium (Na+). levels.In this current study, the results of the kidney parameters indicated no significant increase in serum urea, creatinine, chloride, potassium, and bicarbonate. The results correlate to the findings of Freitas and others [37] that creatinine clearance or urinary and plasma concentrations of Na+, K+, Ca2+, oxalate, phosphate and uric acid were unaffected by oral administration of 1.25 mg/ml/day. Pucci and others [38]also reported no significant change in urinary serum levels of total blood count, creatinine, uric acid, sodium, potassium, calcium, urine volume and pH but indicated a significant increase in urinary potassium from 50.5±20.4 to 56.2±21.8 mg/24-hour. This contrasts with the findings of this current study where the amount of K+ was insignificantly reduced from 7.3 to 6.5 mmol/L. In another finding, Manjrekar and others [39] demonstrated the antioxidant and hepatoprotective activity of P. ninuri on CCl4 induced hepatotoxicity with associated negative effects on kidney and testes where histopathology of both organs revealed tubular damage and degeneration as well as decreased amount of mature spermatozoa respectively. The authors reported a significant reduction in the activities of alanine transaminase, aspartate transaminase, and alkaline phosphatase enzymes in rats treated with P. niruri after CCl4 injection. Moreso, Giribabu, and others [4] concluded that P. niruri helps to preserve near normal kidney function and prevents histopathological changes via ameliorating oxidative stress, inflammation, fibrosis and apoptosis while enhancing proliferation of the kidney in diabetes mellitus (DM) which might be attributed to the content of its antioxidants. Giribabu and others [35] assert that P. niruri leaf extract can protect the kidney from oxidative stress induced by diabetes due to the presence of polyphenols.

Table 4: Effect of aqueous leaf extract of Chanca piedra on kidney function indices in albino rats

Doses (mg/kg)	Urea (mmol/L)	Creatinine (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)
Group 1 (Control)	7.4±0.14	95.5±14.48	143±1.41	111±1.41	7.3±0.28	23±1.41
Group 2 (200)	7.3±0.13	97.5±14.44	145±1.82	112±1.82	8.75±0.21*	23±2.82
Group 3 (400)	7.05±021	100±15.51	150±1.41*	110±1.48	7.3±0.14	22±1.14
Group 4 (800)	7.5±1.01	94.5±12.02	151±1.84*	114±1.72	6.5±0.35	23.5±2.12

The values are presented as mean ± SD, n=4. * Indicate significant difference when compared to control (p<0.05).

5.Conclusion

In conclusion, this study has generally found that the rats were unaffected by the aqueous extract of *Phyllantusniruri*at the dosages administered. However, complete safety of the extract could not be established for the significant increase in the level of K^+.

6. Disclosure and Conflict of interest

The authors declare that they have no conflicts of interest.

References

[1] R. Khandia et al., "Evaluation of the ameliorative effects of Phyllanthus niruri on the deleterious insecticide imidaclopridin the vital organs of chicken embryos", *J Ayurveda Integr Med*,https://doi.org/10.1016/j.jaim.2019.03.003

[2] R. Thonn andP.C.Schum,"The protein fraction of *Phyllahntusniruri* plays a protective role against acetaminophen induced hepatic disorder via its antioxidant properties", *Phytother Res*, vol. 7, pp. 595-601, 2006.

[3] S. Kieley, R. Dwivedi and MMonga,"Ayurvedic medicine and renal calculi", *J. Endourol*, vol. 22, no. 8,pp. 16-13, 2008.

[4] N. Giribabu, K. Karim, E. K.Kilari,and N. Salleh, "Phyllanthus niruri leaves aqueous extract improves kidney functions, ameliorates kidney oxidative stress, inflammation, fibrosis and apoptosis and enhances kidney cell proliferation in adult male rats with diabetes mellitus", *Journal of ethnopharmacology*, vol. 205, pp. 123-137, 2017.

[5] M.A. Boim, I.P. Heilberg and N. Schor,"*Phyllahntusniruri* as a promising alternative treatment for nephrolithiasis,"*IntBraz J Urol.*,vol. 36, no. 6,pp. 657-64, 2010.

[6] Narendra, K., Swathi, J., Sowjanya, K. and Satya, A.K., "Phyllanthus niruri: a review on its ethno botanical, phytochemical and pharmacological profile," *Journal of Pharmacy Research*, vol. 5, no. 9, pp.4681-4691, 2012.

[7] C.Alves, da Maciel, D. E. Pereira, R. de Cássia, de A. Bidô, J. C. R. Freitas, C. P. Fernandes, dos Santos, and J. K. B. Soares, "Effects of the Aqueous Extract of Phyllanthus niruriNiruri Linn during Pregnancy and Lactation on Neurobehavioral Parameters of Rats' Offspring." *Journal of Ethnopharmacology*, vol. 270, p. 113862. https://doi.org/https://doi.org/10.1016 /j.jep.2021. 113862.

[8] A. O. Lateef, and B. A. Edwin, "Solvent Extracts of Different Parts of Ghanaian Phyllanthus Niruri Show Potent Growth Inhibition against Six Human Pathogenic Bacteria in vitro". *International Journal of Pharmacy and Pharmaceutical Sciences*, vo.l6, no. 7, 2014.

[9] R. Djaja, R. Wahyudianingsih, M. Elisabeth, B. Maesaroh, and W. Widowati, "Antioxidant Activity of Phyllanthus Niruri Extract, Rutin and Quercetin." *Indonesian Biomedical Journal*, vol. 9, no. 2, 84–90, 2017. https://doi.org/10.18585/inabj.v9i2.281.

[10] S. Kumar,"*Phyllanthusamarus*Schum. AndThonn.as Herbal Medicine: Ethnobotany, Phytochemistry, and Pharmacology Aspects,*In* Botanical Leads for Drug Discovery*,"Springer*,

[11] N. Kaur, B. Kaur, and G. Sirhindi," Phytochemistry and pharmacology of Phyllanthus niruri L.: a review," *Phytotherapy research*, vol. 31, no. 7, pp. 980-1004, 2017.Singapore. pp. 179-199. 2020.

[12] F. Claudio, A. Chiavaroli, P. Angelini, R.Venanzoni, G. A. Flores, L. Brunetti, M. Petrucci, M.Politi, L.Menghini, S. Leone, L.Recinella, G. Zengin, G. Ak, M. Di Mascio, F. Bacchin, and G. Orlando."Phenolic Content and Antimicrobial and Anti-Inflammatory Effects of Solidagovirga-aurea, Phyllanthusniruri, Epilobiumangustifolium, Peumusboldus, and Ononisspinosa Extracts" Antibiotics, vol. 9, no. 11,2020.https://doi.org /10.3390 /antibi otics9110783

[13] L. Taylor,"*Technical Data Report for CHANCA PIEDRA* "*Stone Breaker" (Phyllanthus niruri)", Preprinted from Herbal Secrets of the Rainforest, 2nd edition*,2003, Sage Press, Inc.

[14] K. H. Krishnamurthy,Bhumiamalaki, "Phyllanthus. Namah," *The Journal of Integral Health*, vol. 22, no. 3, 2014.

[15] de M. Mello and P.Boccolini, "SiqueiraBoccolini, C. Prevalence of complementary and alternative medicine (CAM) use in Brazil", *BMC Complement Med Ther*, vol. 20, no. 51, 2020, https://doi.org/10.1186/s12906-020-2842-8

[16] M. R. Kachmar et al., "Traditional Knowledge of Medicinal Plants Used in the Northeastern Part of Morocco," *Evidence-Based Complement. Altern.Med*., vol. 2021, p. 6002949, 2021,doi: 10.1155/2021/6002949.

[17] T. M. Cerqueira, A. C. de CarvalhoCorreia, R. V dos Santos, R. P. L. Lemos, S. A. da Silva, and E. Barreto, "The Use of Medicinal Plants in Maceió, Northeastern Brazil: An Ethnobotanical Survey,"*Medicines* , vol. 7, no. 2. 2020, doi: 10.3390/medicines7020007.

[18] N. A. Sukri, "Effect of different types of solvent on extraction of phenolic compounds from Cosmos caudatus" (Doctoral dissertation, UMP), 2012.

[19] A. M. Bode, and Z. Dong, "Toxic phytochemicals and their potential risks for human cancer," *Cancer prevention research (Philadelphia, Pa.)*, vol. *8, no*. 1, pp 1–8, 2015, https://doi.org/10.1158/1940-6207.CAPR-14-0160.

[20] G.E Trease, and W.C Evans, "*Trease and Evans pharmacognosy*", 14th edition *W.B Ssuvnder company limited*, London, pp 191-293, 1996.

[21] W.C Evans, "Trease and Evans Pharmacognosy", Harcourt Brace and company. *Asia pvt. Ltd*., Singapore, 1997.

[22] C.K Kokate, "*Practical pharmacognosy*" (4th edition), *VallabhPrakashan Publication*, New Delhi, India, 1999, Pp23.
[23] A. Sofowora, "Medicinal plants and traditional medicine in Africa," *Spectrum books LTD*, Ibadan, Nigeria, P289, 1993.
[24] C. S. Ezeonu, and C. M Ejikeme, "Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods", *New Journal of Science*, 2016, pp 1–9, https://doi.org/10.1155/2016/5601327
[25] S. Reitman, S Frankel, "Glutamic – pyruvate transaminase assay by colorimetric method", Am. J. Clin. Path, 28: 56, 1957.
[26] H. L. Rosenthal, "Determination of Urea in Blood and Urine with DiacetylMonoxime", *Analytical Chemistry*, vol. *27, no.* 12, pp 1980–1982, 1955,https://doi.org /10.1021/ac60108a039.
[27] A. Kaplan, L. L Szabo, "*Clinical Chemistry: Interpretation and Techniques, Lea and Febiger," Philadelphia*, 1983, p34.
[28] A. Pauss, A. Roza, M.-J. Ledrut, H. Naveau& E.-J. Nyns, "Bicarbonate determination in complex acid-base solutions by a back-titration method", *Environmental Technology*, vol. 11, no. 5, pp 469-476,1990, DOI: 10.1080/09593339009384887
[29] D. Lorke, "A new approach to practical acute toxicity testing," *Arch Toxicol*, vol. 54, pp 275-87, 1983.
[30] M. Mathur, R. Sharma, J. Sharma, R.Pareek, and R. Kamal, "Phytochemical screening and antimicrobial activity of Phyllanthus niruri Linn", *Elixir Appl Botany*, vol. 46, pp 8487–8489, 2012.
[31] A. D.Njoroge, B. Anyango, S.F. Dossaji,"Screening of Phyllanthus species for antimicrobial properties," *ChemSci J,vol*. 56, no. 1–11, 2012.
[32] N. Aarthi, and K. Murugan, "Antimalarial activity and phytochemical screening of ethanolic leaf extract of Phyllanthus niruri and Mimosa pudica" *IJPRD*, vol. 3, no. 3, p 24, 2011.
[33] G. A. Asare, P.Addo, K.Bugyei, B.Gyan, S.Adjei, L. S.Otu-Nyarko, and A. Nyarko, "Acute toxicity studies of aqueous leaf extract of Phyllanthus niruri", *Interdisciplinary toxicology*, vol. 4, no.4, p. 206, 2011.
[34] V. G. Paula, L. L. Cruz, L. B. Sene, T. B. Gratão, T. S. Soares, R. Q.Moraes-Souza, ... & G. T. Volpato,"Maternal-fetal repercussions of Phyllanthus niruri L. treatment during rat pregnancy", *Journal of Ethnopharmacology*, vo. 254, pp112728, 2020.
[35] N. Giribabu, P. V. Rao, K. P. Kumar, S.Muniandy, R. S. Rekha,and N.Salleh, "Aqueous extract of Phyllanthus niruri leaves displays in vitro antioxidant activity and prevents the elevation of oxidative stress in the kidney of streptozotocin-induced diabetic male rats. Evidence-Based Complementary and Alternative Medicine, 2014.
[36] M. I. Ezzat, M. M. Okba, S. H. Ahmed, H. A. El-Banna, A. Prince, S. O. Mohamed, et al.,"In-depth hepatoprotective mechanistic study of Phyllanthus niruri: In vitro and in vivo studies and its chemical characterization" *PLoS ONE*, vol. 15, no. 1, pp e0226185, 2020,https://doi.org /10.1371 /journal.pone.0226185.
[37] A. M.Freitas, N. Schor, and M. A. Boim, "The effect of Phyllanthus niruri on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation." *BJU international*, vol. 89, no. 9, 829-834, 2002.
[38] N. D. Pucci, G. S. Marchini, E. Mazzucchi, S. T. Reis, M.Srougi, D. Evazian, and W. C. Nahas,"Effect of Phyllanthus niruri on metabolic parameters of patients with kidney stone: a perspective for disease prevention", *International braz j urol*, vol. 44, no. 4, pp 758-764, 2018.
[39] A. P. Manjrekar, V.Jisha, P. P. Bag, B.Adhikary, M. M.Pai,A.Hegde, M.Nandini,"Effect of Phyllanthus niruri Linn. treatment on liver, kidney and testes inCCl4 induced hepatotoxic rats,"2008.