



Research Article

Anti-Inflammatory Activity of The Methanol Extracts of *Cissus Populnea* GUILLS & PERS and *Cissus Arguta* HOOK. F.

Ogunmefun S.A.,¹ *Ajayi T.O.¹, Moody J.O.¹

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Accepted: October 14, 2022

Abstract

Inflammation, characterized by pain, heat, swelling, redness, and loss of body function, is a living tissue's initial response to injury. There are ethno-botanical claims made about these plants for their wound-healing abilities which have not been scientifically validated. Therefore, this study was conducted to examine the anti-inflammatory activity of the two related *Cissus* species. The plant samples (leaf, stem and root) were dried, powdered and extracted with methanol (100%) and concentrated *in vacuo* and evaporated to dryness at room temperature. The anti-inflammatory activity of the plant extracts was evaluated using the *in vitro* protein denaturation and carrageenan-induced foot pad assay in chick, Diclofenac sodium as the reference and saline as control. Anti-inflammatory effects were observed in a dose-dependent manner. The phytochemical screening performed on the extracts showed the presence of phenols, flavonoids, tannin, anthraquinone, glycosides, saponin and alkaloid. The leaf extract of *C. arguta* and the stem extract of *C. populnea* inhibited protein denaturation with a value of 64.46 ± 0.10 and 53.34 ± 0.04 at 3 mg/mL respectively compared to the control group and diclofenac (52.86 ± 0.00) at 3 mg/mL. All concentrations of extract tested; 10, 30, 100 mg/kg) for *in vivo* study had a significant effect in reducing total edema (AUC) ($P < 0.01$), and in most case they performed better than diclofenac. However, root extracts of *C. populnea* at 30 mg/kg (7.13 ± 1.06) had better result in inhibiting edema in chicks than *C. arguta* extracts at 30 mg/kg (5.64 ± 0.05). The plants exhibited anti-inflammatory activity which can be of great pharmacological importance.

Key Words: Anti-inflammatory, *Cissus arguta*, *Cissus populnea*, Protein denaturation, Carrageenan

INTRODUCTION

Inflammation is part of the body's defense mechanism and can be thought of as a living tissue's first response to injury. It is a complex process in which the immune system recognizes harmful and foreign stimuli, eliminates them and initiates the healing process. Inflammation can be either acute (short-term) or chronic (long-term) (Michels da Silva *et al.*, 2019, Fritsch and Abreu, 2019) and can be caused by infection, physical injury, or autoimmune diseases. The five cardinal signs of inflammation can include redness, heat, pain, swelling and loss of function of any part of the body (Ferrero-Miliani *et al.*, 2007). When any body part experiences injury, the arterioles dilate and the flow rate of blood increases and move to the injury site, causing the injured area to become red (Verma 2016). While aging, unbalanced diet, low sex hormones, and smoking are some of the potential factors that trigger inflammation (Franceschi and Campisi 2014), chronic inflammation has been associated with the occurrence of hypertension, diabetes, cancer, atherosclerosis, and rheumatism which has been said to be age-related diseases (Freund *et al.*, 2010). Currently available anti-inflammatory drugs block the activity of enzymes and relieve symptoms, but they have side effects. Therefore, it is important to look for anti-inflammatory drugs with fewer side effects.

Cissus populnea (Figure 1a), generally named Okoho (Idoma and Igala), Ogbolo ajara (Yoruba), and Dafaaraa (Hausa) (Burkill, 2000), belong to the Vitaceae family. It has been recognized for its diuretic (Belmain *et al.*, 2000), antibacterial (Kone *et al.*, 2004), antifungal (Kone *et al.*, 2004), antiscikling (Moody *et al.*, 2003, Adebayo *et al.*, 2015) antioxidant, anti-infertile (Ojekale *et al.*, 2006) and anti-trypanosomal activities (Atawodi *et al.*, 2002). Direct analysis of the stem bark of *Cissus populnea* is particularly rich in carbohydrates, crude fiber (Achikanu *et al.*, 2020) and vitamins (Brett 2013). *Cissus arguta* (Figure 1b) on the other hand is a herbaceous climber of the Vitaceae family. Traditionally, the plant has been used to treat fevers, coughs, chest pains, inflammation of the lymph nodes, blockages in blood vessels, rashes, boils, wounds, skin and sexually transmitted infections, body aches, and bone-related diseases and disorders (Embido, 2000). The plant contains phytochemicals such as alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, steroids, terpenoids and eugenols in both the leaf and stem (Dickson *et al.* 2012). The plant has also been described for its antibacterial, antifungal, and wound healing activities (Dickson *et al.* 2012). The anti-inflammatory potential of extracts of the leaf, stem and root of *Cissus populnea* and *Cissus arguta* was evaluated.

MATERIALS AND METHODS

Collection, identification and preparation of plant materials: The leaf, stem and root of *Cissus populnea* were collected in Eruwa Village, Ibarapa East Local Government, Oyo State, Nigeria in August 2021, while the leaf and stem of *Cissus arguta* were collected along the Amina way within the University of Ibadan, Oyo State, Nigeria, were collected September 2021. Plants were identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State. Samples of the plant material have been deposited in the Herbarium of the Department of Pharmacognosy, University of Ibadan (DPHUI), Oyo State.



Fig 1a: Habit photograph of *Cissus populnea* Guils & Pers



Fig 1b: Habit photograph of *Cissus arguta* Hooke. F

Extraction of Plant Material: Foliage plant samples were washed, air dried for 14 days; the stem and root samples for 49 days at the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Oyo State. The plant samples were pulverized by grinding using a grinder at the Department of Agronomy, University of Ibadan, Ibadan, Oyo State. A weighed amount (200 g) of the powdered samples was extracted with 100% distilled methanol using the maceration method for one week. The macerated samples were then filtered, concentrated in vacuo using a Rotary evaporator and the filtrate was evaporated to dryness at room temperature at the Department of Pharmacognosy Laboratory, University of Ibadan, Ibadan, Oyo State.

Chemical and reagents: Analytical grade chemicals and reagents used in this study include; Methanol (BDH Chemicals Ltd. England), Sodium hypochlorite solution (Tolarem Africa Enterprise), Distilled water, Ferric chloride, Dichloromethane, Methanol, Acetic Acid (Sigma-Aldrich, Co., USA), Dragendorff's (Hopkins and Williams, Ltd. England), Mayer's Reagent (Hopkins and Williams, Ltd. England), Wagner's Reagent (Hopkins and Williams, Ltd. England), Nitric acid, Picric Acid, Sodium Hydroxide, 10% ammonium hydroxide, 10% Lead acetate solution, Acetronitile, Sulphuric acid, Perchloric acid, methyl red, Phosphate buffer saline (PBS). Apparatus used in this study include Drying oven (Haier Thermocool), Weighing balance (Kebro BL-P1D/20001), Electric digital caliper, Beakers (Pyrex), Flat bottom flask (Pyrex), Glass funnel (Pyrex), Measuring cylinder (Pyrex), Test tubes (Pyrex), Test tube rack, Aluminium foil paper (Tower), Cotton wool, Water bath

(Searchtech Instruments), Test tube holder, Magnetic stirrer (Gallanamp), Silica Crucible (England), Refrigerator (Haier Thermocool), Forceps, TLC plates, Filter papers (Whatman's No 1 filter paper), Pencils, Ruler, Hand gloves, Syringe, burner, pH Meter (Search Tech Instruments), Cuvettes and UV-VIS Spectrophotometer (Spectrumlab Model 725).

PHYTOCHEMICAL ANALYSIS: Phytochemical screening was performed on the powdered samples of *Cissus populnea* using standard methods (Gunavathy SK, Sherine HB, 2019, Evans WC, 2009) to screen for the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones and others.

Protein Denaturation Assay: The in vitro anti-inflammatory assay was performed using a total volume of 5 mL reaction mixture prepared by dissolving 0.2 mL egg albumin (fresh egg from a chicken), 2.8 mL phosphate buffered saline (PBS), adjusting its pH to 6.4, and 2 mL of the various concentrations of 1, 2 and 3 mg/mL extract solutions. A volume of 2 mL of diclofenac sodium (reference drug) with different concentrations similar to those of the extracts was prepared and 2 mL of double distilled water solution served as a negative control. The experiment was performed in triplicate. The mixtures were incubated at 37°C in an incubator for 15 minutes, after which they were heated in a water bath at 70°C for 5 minutes to induce denaturation. A UV-Vis spectrophotometer was used to measure the absorbance of the solutions at their respective concentrations at 660 nm. The negative control was adjusted to an absorbance of 0.00 nm. The procedure was independently replicated three times to obtain three separate sets of data for the analysis.

The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ = Absorbance of negative control, A₁ = Absorbance of test solution.

EVALUATION OF IN VIVO ANTI-INFLAMMATORY ACTIVITY

Experimental Animals: 7-day-old chicks (280 g – 420 g) were obtained from the popular Akobo-ojurin market in Ibadan, Oyo State. They were housed in approved laboratory environments (temperature 25.2°C, 12 h light-dark cycle), provided with food (starter feed, Ibadan, Nigeria) and clean water *ad libitum*. The chicks were tested after 48 hours of acclimatization. The sample size design was such that 6 chicks (n=6) were grouped together.

Dose preparation and administration: The dose preparation and administration of the *in vivo* anti-inflammatory test was carried out such that three doses (10, 30 and 100 mg/kg) were prepared for each of the five extracts using normal saline as the vehicle. The standard drug diclofenac was also prepared at 10 mg/kg using normal saline. The actual doses of the extracts and the standard drug were calculated using the individual weights of the chick while administration was intraperitoneal.

In one group, two chicks received one of the doses of each extract.

Carrageenan-induced foot pad edema in chicks: The anti-inflammatory properties of the extracts were assessed using the modified model of Roach and Sufka (2003) by Ainooson et al., (2012) while diclofenac served as the reference drug. Chicks were induced with 10 µL of 2% carrageenan in the sub-plantar region of their right footpad. Foot volume was measured with an electronic digital caliper before injection and at hourly intervals for 6 hours after injection. At different time interval, the edema component of inflammation was quantified by measuring the difference in foot volume before carrageenan injection. The extracts were administered at 10, 30 and 100 mg/kg by sub-plantar injection and intraperitoneal injection for diclofenac (positive control). Control animals received only saline, which served as vehicle. The increase in foot volume was calculated using the equation below,

$$\% \text{ increase in foot volume} = \frac{\text{Foot volume at time t} - \text{Foot volume at time 0}}{\text{Foot volume at time 0}} \times 100$$

Total foot volume for each treatment group was calculated in arbitrary unit as the area under the curve (AUC). Percentage inhibition of edema for each treatment group was then determined as follows:

$$\% \text{ inhibition of edema (AUC)} = \frac{\text{AUC control} - \text{AUC treatment}}{\text{AUC control}} \times 100$$

Table 1:

Phytochemical screening result for extracts of *Cissus populnea* and *Cissus arguta*

Parameters	<i>Cissus populnea</i>			<i>Cissus arguta</i>	
	Leaf	Stem	Root	Leaf	Stem
Saponin	++	+	+++	++	+
Coumarin	-	-	-	+	-
Steroid	-	-	-	+	-
Sterols	-	-	-	+	-
Alkaloid	++	++	+	++	+++
Anthraquinone	++	++	+	-	-
Flavonoid	+++	++	+++	++	+
Phenol	+++	+	-	++	+
Tanin	+++	+	-	+	-
Glycoside	++	+	-	+	+

Key: + = Present ++ = Moderately Present - = Absent

Protein denaturation assay: The five extracts screened for anti-denaturation activity using protein egg albumin had remarkable activity. From the study, it was observed that the leaf extract of *C. arguta* and the stem extract of *C. populnea* showed remarkable anti-inflammatory activity with a percentage inhibition value of 64.46±0.10 and 53.34±0.04 at 3 mg/mL compared to the untreated control group and diclofenac (52.86±0.00) at 3 mg/mL. However, diclofenac (reference drug) performed better than leaf, stem and The percentage inhibition of the stem extract of *Cissus populnea* was slightly higher 53.34±0.03 than reference drug

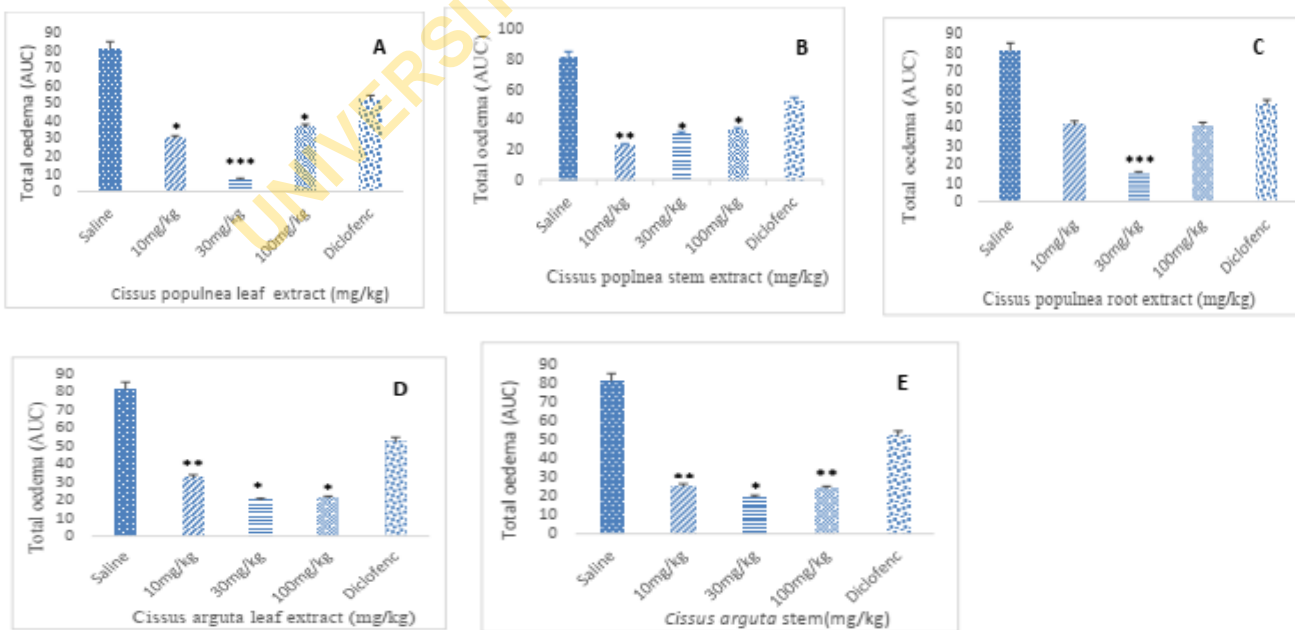
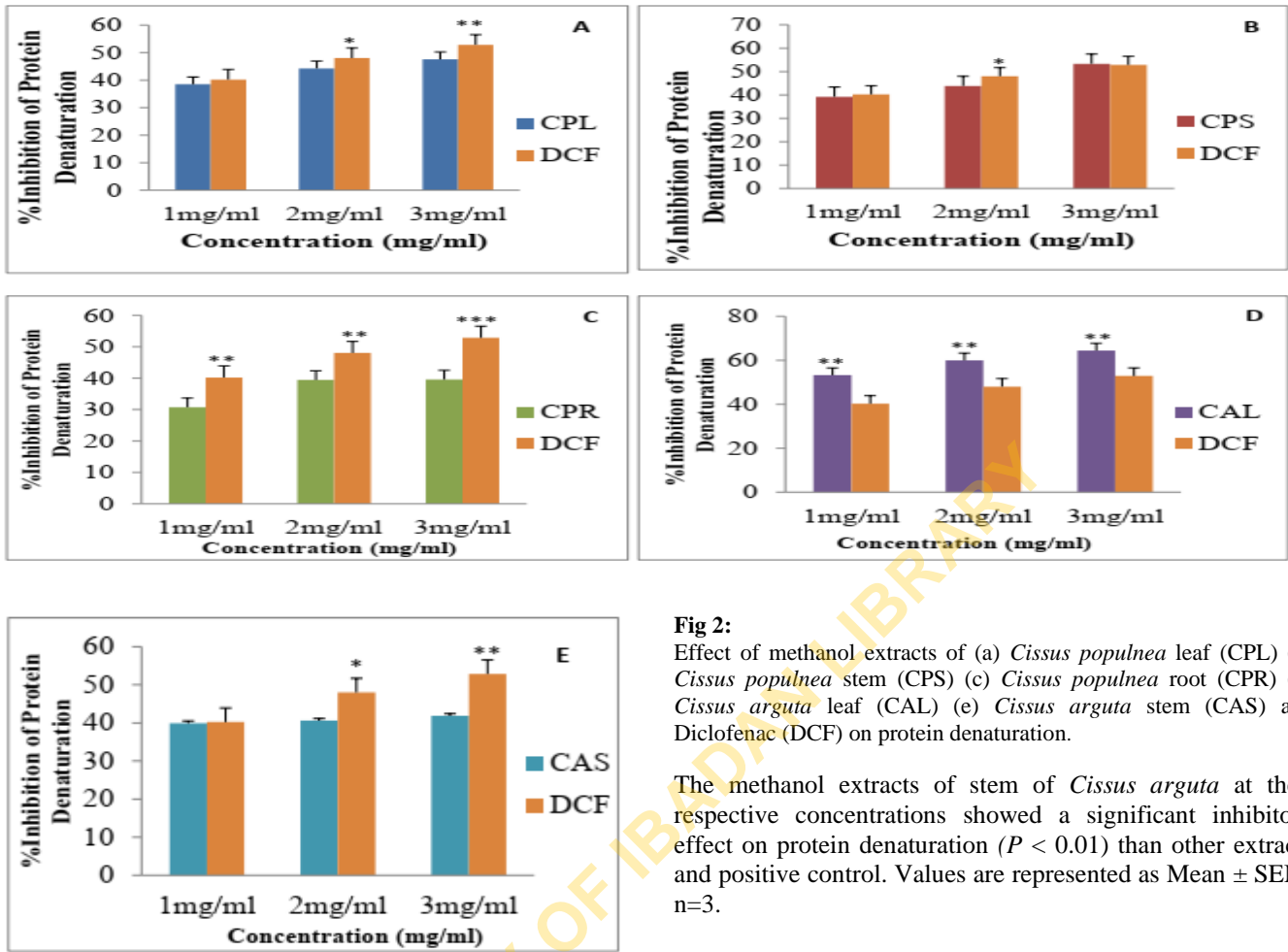
Where AUC = Area under the Curve, expressed as % inhibition of edema

STATISTICAL ANALYSIS: The data obtained were expressed as SEM means of values obtained in triplicate from three independent experiments. The evaluation was performed using a one-way analysis of variance (ANOVA). A P-value less than 0.01 (P<0.01) was considered statistically significant. The data were analyzed with the SPSS software.

RESULTS

Phytochemical screening: The phytochemical screening showed that saponins, alkaloids and flavonoids were present in all test extracts (Table 1). Alkaloids were found in abundance in the stem extract of *Cissus arguta* (CAS), while flavonoids, tannins and phenols were abundant in the leaf extract of *Cissus populnea* (CPL). Of all the extracts, *Cissus populnea* root extract had the highest level of saponin but phenols, tanins and glycoside was absent. The leaf extract of *Cissus arguta* had coumarin, steroids and sterols in trace amount. All the selected parts of *Cissus populnea* showed positive test for anthraquinone while *Cissus arguta* showed negative result for anthraquinone.

(52.86±0.19) at 3 mg/mL. The root extract of *Cissus populnea* had the lowest activity with percentage inhibition ranging from 30.74±0.21 to 39.60±0.47 at concentration of 1 - 3 mg/mL. The anti-inflammatory activity of leaf extract of *Cissus arguta* as observed in figure 2 was remarkably better than the reference drug across all concentrations. At 1 mg/mL, the stem extract of *Cissus populnea* and diclofenac were showing almost similar percentage inhibition but the inhibitory activity was later in favour of the reference drug at 2 and 3 mg/mL with 48.02±0.73 and 52.86±0.02 respectively.



Diclofenac sodium and normal saline served as positive and negative control respectively. Data are expressed as mean \pm SEM, (n=5) $P < 0.01$. One-way ANOVA was used for statistical analysis followed by Dunnett's post hoc test.

Carrageenan-Induced Edema in Chicks: Intraperitoneal administration of carrageenan resulted in a marked increase in the paw size of the chicks. In control chicks, maximum inflammation was observed at two hours, followed by a slight decrease in inflammation at four and six hours. Intraperitoneal administration of diclofenac (the positive control) and subplantar administration of the extracts reduced inflammation in the chicks significantly and faster than the negative control group. In general, all the extracts that were examined in this study significantly reduced inflammation. One-way ANOVA data treatment followed by Dunnett's post hoc test showed that the effect of extracts on edema was significant ($p < 0.01$). All tested extract concentrations; 10, 30, 100 mg/kg had significant effect in reducing inflammation ($p < 0.01$), and in most case they performed better than the reference drug, diclofenac. However, a medium dose of 30 mg/kg of the leaf and root extracts of *Cissus populnea* had a great significant effect in reducing edema in the chicks when compared to other extracts in their respective concentrations. It was also observed that a medium dose of 30 mg/kg of all the extracts showed significant reduction of edema induced by carrageenan.

DISCUSSION

In the inflammatory response, leukocytes and mast cells are recruited to the damaged regions, leading to a respiratory burst as a result of increased oxygen uptake and therefore enhancing the production and release of reactive oxygen species (ROS) in the damaged or injured area (Coussens and Weber, 2002). From this study, it was observed that test extracts of *C. populnea* and *C. arguta* showed concentration-dependent anti-inflammatory activity. A similar dose-dependent anti-inflammatory pattern was observed in previous studies on inhibition of protein denaturation (Akoto et al., 2020, Bensaad et al., 2021). Of all the extracts, *Cissus arguta* leaf extracts (2 and 3 mg/mL) showed the highest inhibitory effect (60 and 64.46%) followed by *Cissus populnea* stem (53.34%) at 3 mg/mL. The anti-inflammatory effect of all concentrations of the leaf extract of *C. arguta* was significantly higher ($P < 0.01$) than diclofenac. The significant anti-inflammatory effects may be due to the presence of phytoconstituents like phenols, flavonoids, glycoside and alkaloids as indicated by the phytochemical analysis carried out in this study. Previous studies have shown that extracts containing promising bioactive phytoconstituents possess antioxidant and anti-inflammatory activity (Akoto, et al., 2020).

When carrageenan was induced in the chicks at the early stage, redness, swelling, pain and weakness was observed indicating the presence of inflammation. Hence, this change in body response of the chicks to carrageenan induction may be as a result of release of various anti-inflammatory mediators as indicated by Abdulkhaleq et al., (2018). Although diclofenac showed anti-inflammatory activity according to the results of this study, all extracts at 10, 30 and 100 mg/kg had an anti-inflammatory effect as they were able to reduce the overall edema of the chicks. However, the anti-inflammatory activity of *C. populnea* leaf and root extracts at 30 mg/kg was superior

to that of *C. arguta* extracts at 30 mg/kg. *Cissus populnea* stem extract showed a significant inhibitory effect on the reduction of edema in chicks at 10 mg/kg when compared to other extracts at same concentration. Also, 100 mg/kg of *Cissus arguta* leaf extract performed better than other extracts at same concentration. It can thus be inferred that the extract that significantly inhibit inflammation by reducing edema level at a lower dose (10 mg/kg) is more potent than the extract that inhibit inflammation at the highest dose of 100mg/kg. Potency is an expression of the activity of a drug in terms of the concentration or amount of drug required to produce a defined effect. According to Berrouet et al., 2020, the amount of drug necessary to produce the definite effect is related to IC50. Also, the lower the IC50 value the more potent the drug (Meyer et al., 2019). It was generally observed that there was a significant anti-inflammatory effect of all extracts at 30 mg/kg which was a moderate dose. Based on our results, there is a possibility that a 30 mg/kg dose of these extracts is likely to produce the desired anti-inflammatory effect.

The phytochemical screening of *C. populnea* showed the presence of flavonoid, tannin and phenol in abundant amount compared to *C. arguta*. Gonzalez-Gay and Gonzalez-Juanatey (2014) earlier reported that flavonoids and phenols inhibit enzymes and are mediators of the inflammatory process.

CONCLUSION

In summary, the result obtained from biological assay indicates that all the samples of the selected plant species had anti-inflammatory activities. At the end of this study, it was revealed that test extracts of *C. populnea* and *C. arguta* showed concentration-dependent anti-inflammatory activity in vitro while a significant anti-inflammatory effect was observed in all extracts at a moderate dose of 30 mg/kg. Therefore, these plant species possesses anti-inflammatory activities and hence can be put forward for drug discovery. In addition, further toxicological studies and identification of secondary metabolite(s) responsible for these activities would be done

REFERENCES

- Abdulkhaleq, L. A., M. A. Assi, R. Abdullah, M. Zamri-Saad, Y. H. Taufiq-Yap, & M. N. M. Hezme. 2018. The crucial roles of inflammatory mediators in inflammation: A review. *Vet. World*, 11(5): 627.
- Achikanu C. E., O. N. Ani, and E. I. Akpata. 2020. Proximate, vitamin and phytochemical composition of *Cucumis metuliferus* seed. *Int.J.Food Sci.and Nut.* 5(3):20-24.
- Adebayo E. M., A. A. Adeyemi, O. O. Omotade, F. A. Fasola, T. O. Ajayi, F. A. Attah, T. I. Famojuro, L. Adebisi, and J. O. Moody. 2015. Antisickling Activity of the Fresh and Dried Roots of *Cissus populnea* Guill. Et Perr (Vitaceae). *Nig. J. Nat.Prod. Med.* (19): 134-138.
- Adedapo, A. A, Falayi O. O and Oyagbemi O. A, 2015. Evaluation of the analgesic, anti-inflammatory, antioxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed *Moringa oleifera* in some laboratory animals. *J.Basic Clin. Physio. And Pharmaco.*5:491-499

- Ainooson G.K., G. Owusu, E. Woode, C. Ansah, K. Annan. 2011. *Trichilia monadelpha* bark extracts inhibit carrageenan-induced foot-oedema in the 7-day old chick and the oedema associated with adjuvant-induced arthritis in rats. *Afr. J. Trad. Compl. Alt. Med.* 9(1):8-16.
- Atawodi S. E., D. A. Ameh, S. Ibrahim, J. N. Andrew H. C. Nzelibe, E. O. Onyike, K. M. Anigo, E. A. Abu, D. B. James, G. C. Njoku, A. B. Sallau. 2002. Indigenous knowledge system for treatment of Trypanosomiasis in Kaduna state of Nigeria. *J. Ethnopharm.* 79(2):279-282.
- Belmain S. R., P. Golo, H. F., Andan, H., Atarigiya, F. A., Chare and P. Carr. 2000. Toxicity and repellency of ethnobotanicals used in Ghana as post-harvest protectants, in Abstracts of presentations on selected topics at the XIVth International Plant Protection Congress (IPPC). *Phytoparasitica*, 28(1):87 – 90
- Bogdanov S., T. Jurendic, R. Sieber and Gallmann P. 2008. Honey for nutrition and health: a review. *J. America. Coll. Nutri.* 6:677–689.
- Burkill, H.M. 2000. The useful plant of west tropical Africa, Vol.5 Royal Bot.Gard. Kew, pp 296-297.
- Chung H. J., H. S Lee., and Shin J. S. 2010. “Modulation of acute and chronic inflammatory processes by a traditional medicine preparation GCSB-5 both in vitro and in vivo animal models,” *J. Ethnopharma.* 3:450–459.
- Dickson, Udeme J. Igbe, Igbodaro and Edema, Mary Olire, 2012. Antibacterial and antifungal activity and Bone healing Potency of methanol extract of the *Cissus arguta* Hook F. *Nig.J. Chem. Res.* 17:1 – 11.
- Fard M. T., P Arulselvan, G Karthivashan, S. K Adam and Fakurazi S. 2015. Bioactive extract from *Moringa oleifera* inhibits the pro-inflammatory mediators in Lipopolysaccharide stimulated macrophages. *Pharmacog. Magazine.* 4:556–563.
- Ferrero-Miliani L., O. H. Nielsen P. S. Andersen., and S. E. Girardin. 2007. "Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation". *Clin. Exp. Immuno.* 147 (2): 227–35.
- Franceschi C. and J. Campisi. 2014. Chronic inflammation and its potential contribution to age-associated diseases. *J. Gerontology Appl. Bio. Sci. and Med. Sci.* 69:4-9.
- Freund A, A.V., Orjalo, Y., Desprezp., and J. Campisi. 2010. Inflammatory networks during cellular senescence: Causes and consequences. *Trends in Mol. Med.* 16:238-46.
- Fritsch, J., and M.T. Abreu. 2019. The Microbiota and the Immune Response. What Is the Chicken and What Is the Egg? *Gastrointest Endoscop Clin.* 29(3):381-393.
- González-Gay M. A., and C. González-Juanatey. 2014. Inflammation and lipid profile in rheumatoid arthritis: bridging an apparent paradox. *Ann.Rheumatic Diseases.* 73(7):1281–1283.
- Karthik K., B. Kumar, K.R.V. Priya, S.K. Kumar, and R.S.B. Rathore. 2013. “Evaluation of anti-inflammatory activity of *canthium parviflorum* by in-vitro method,” *India J. Res. in Pharm.. and Biotech.* 5674:2320–2322.
- Kone W.M., K.K., Atindehou, C. Terreaux K. Hosettman, D. Traore, and M. Dosso. 2004. Traditional medicine in north Cote-d’Ivoire: screening of 50 medicinal plants for antibacterial activity. *Ethnopharmacol Bul.* 93(1): 43 – 49.
- Meyer C. T., D. J. Wooten, B. B. Paudel, J. Bauer, K. N. Hardeman and Westover D. 2019. “Quantifying Drug Combination Synergy along Potency and Efficacy Axes”, *Cell Syst.* 8(2):97-108..
- Michaels da Silva D., H. Langer., and T. Graf. 2019. Inflammatory and Molecular Pathways in Heart Failure Ischemia, HFpEF and Transthyretin Cardiac Amyloidosis. *Int. J. Mol. Sci.* 20(9): 2322.
- Mirke N. B., P. S. Shelke, P. R. Malavdkar, and J. N. Jagtap. 2020. In vitro protein denaturation inhibition assay of *Eucalyptus globulus* and *Glycine max* for potential anti-inflammatory activity. *Innov. Pharm. Pharmacotherapy.* 8(2):28-31.
- Moody J.O., O.O. Ojo, O.O. Omotade A.A. Adeyemo, P.E. Olumese, and O.O. Ogundipe. 2003. Anti-sickling potential of a Nigerian herbal formula (ajawaron HF) and the major plant component (*Cissus populnea* L. CPK). *Phytotherapy Research: Int. J. Pharmacol. and Toxicol. Eval. of Nat. Prod. Deriv.* 17(10):1173-117
- Ojekale A. B., O.A. Lawal, A.K. Lasisi, and T.I. Adeleke. 2006. Phytochemistry and spermatogenic potentials of extract of *Cissus populnea* (Guill and Pers) stem bark. *TSW Holistic Health Med.* 1: 176 – 182.
- Sree Kumari, N., M. Yasmin, R. Hussain and M. Babuselvam. 2015. invitro anti-inflammatory and anti-arthritic property of *rhizopora mucronata* leaves. *Int. J. Pharma. Sci. Res.* 6:482-485.
- Sulaiman M. R., E. K Perimal, and Akhtar M. N. 2010. Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice. *Fitoterapia.* 7:855–858.
- Verma S. 2016. Medicinal plants with anti-inflammatory activity. *J. Phytotharam.* 5:157-9.