International Journal of Pharmacognosy and Clinical Research

ISSN Print: 2664-763X
ISSN Online: 2664-7648
Impact Factor: RJIF 8.00
IJPCR 2023; 5(1): 35-43
www.pharmacognosyjournal.in
Received: 19-11-2022
Accepted: 27-12-2022

Gerald Walter Ugodi
Department of Pharmaceutical Chemistry, Enugu State University of Science and Technology, Enugu State, Nigeria

Rejoicing Chijindum Innocenta
Department of Pharmaceutical Chemistry, Enugu State University of Science and Technology, Enugu State, Nigeria

Ugwu Agatha Adora
Department of Pharmaceutical Chemistry, Enugu State University of Science and Technology, Enugu State, Nigeria

Corresponding Author:
Gerald Walter Ugodi
Department of Pharmaceutical Chemistry, Enugu State University of Science and Technology, Enugu State, Nigeria

Phytochemical screening and in vitro evaluation of anti-inflammatory potential of crude extract and fractions of the leaves of *Ficus umbellata*

Gerald Walter Ugodi, Rejoicing Chijindum Innocenta and Ugwu Agatha Adora

DOI: https://doi.org/10.33545/2664763X.2023.v5.i1a.31

Abstract
Ficus umbellata is an evergreen tree species originating from Africa, it is often grown for the leaves which serve traditionally for the treatment of various diseases, including inflammatory diseases. Despite the traditional use of *Ficus umbellata* in the treatment of inflammatory disorders, there is a lack of scientific evidence to support its anti-inflammatory properties. The research aims to evaluate the *in vitro* anti-inflammatory activity of the crude extract of *Ficus umbellata* to provide a potential new source of natural anti-inflammatory agents. The extraction was carried out by cold maceration using methanol. The extract was qualitatively assessed for the presence of major phytochemical constituents using standard analytical phytochemical screening procedure. Acute toxicity study was carried out by Lorke's method and the anti-inflammatory evaluation was done using anti-platelet aggregatory activity and stabilization of human red blood cell membrane tests. The percentage yield was 8.15%. The phytochemical constituents present in the extract were alkaloids, phytosteroids, phenolic compounds, terpenoids, flavonoids, tannins, saponins, steroids, quinones, and triterpenoids. Acute toxicity evaluation indicated no death even at 5000 mg/kg dose. The results showed that the mechanism of anti-inflammatory activity of the crude and fractions of *Ficus umbellata* might be by inhibition of platelet aggregation, but not by stabilizing the red blood cells. The methanol extract and the fractions significantly at ($p< 0.05$) have an inhibitory effect on platelet aggregation in a dose-dependent manner. Furthermore, *it* is not as effective as aspirin in inhibiting platelet aggregation.

Keywords: *Ficus umbellate*, anti-inflammatory, phytochemicals, membrane stabilization, platelet aggregation, leaves

Introduction
Inflammation (from Latin: *inflammation*) is a protective reaction involving immune cells, blood vessels, and chemical mediators and is a component of the intricate biological response of bodily tissues to harmful stimuli, such as pathogens, injured cells, or irritants (Chen *et al*, 2017) [83]. Inflammation serves to remove the original source of cell injury, remove necrotic cells and tissues that have been harmed by both the initial insult and the inflammatory process, and start the repair process for injured tissues (Chen *et al*, 2017) [83]. The five identifying characteristics are heat, pain, redness, swelling, and loss of function (Latin; *calor*, *dolor*, *rubor*, *tumor*, and *functio laesa*). In contrast to adaptive immunity, which is tailored to each disease, inflammation is a general reaction, making it a component of innate immunity (Abbas & Lichtman, 2009) [84]. Inflammation can be acute or chronic (Zhang *et al.*, 2019; Fritsch and Abreu, 2019) [85, 86]. During acute inflammatory reaction, cellular and molecular events reduce imminent injury or infection. This mitigation process contributes to the restoration of tissue homeostasis and resolution of the acute inflammation. Uncontrolled acute inflammation may, however, become chronic, contributing to a variety of chronic inflammatory diseases (Zhou, Hong & Huang, 2016) [81]. Modulation of inflammation with the use of medicinal plants proposed an alternate to conventional therapeutic strategies for numerous ailments, particularly when suppression of inflammation is expected (Fritsch & Abreu, 2019) [86]. The study of natural compounds in consort with pharmacological and ethno botanical information are significant contributions for further improving these traditional

compounds (Tasneem *et al.*, 2019) [68]. It is generally assumed that the active constituents contributing to these protective effects are the phytochemical, anti-inflammatory constituents and minerals (Gill, 2017) [87]. Currently available anti- inflammatory drugs block both enzyme activities and relief symptoms despite they have serious side effects (Verma, 2016) [76]. Therefore it is essential to administer anti- inflammatory drugs with lesser side effects. During the attempt of identification of medicinal plants and their extracts with proven anti- inflammatory activity, studies using *in vitro* assays and *in vivo* models of inflammation have used (Maionea *et al.*, 2015) [41]. There are ethical issues of using the animals in the early stages of drug discovery for inflammatory diseases (Tatti *et al.*, 2012) [69]. *In vitro* studies help to study the cellular response in a closed system where the experimental conditions are maintained (Doke & Dhawale, 2015) [18]. These *in vitro* studies are helpful in developing an understanding of the mechanism of anti- inflammatory activity of herbal constituents (Umar *et al.*, 2010) [72].

Among these herbal resources, *Ficus umbellata* leaves selected for this study belongs to the family Moraceae. It is a beautiful plant with large leaves, ideal to beautify our environment. But it is not only used to decorate, but it also produces exquisite fruits (Arbonnier *et al.*, 2009) [88]. However, it is a tropical tree commonly called "Mewed" in "Guiziga". Its stem barks are used in Cameroonian traditional medicine system for the treatment of many gynecological conditions, such as amenorrhea, dysmenorrhea as well as for menopausal complaints (Arbonnier *et al.*, 2009) [88]. Its leaves are traditionally used to treat hemorrhoids in Benin and used in traditional medicine and pharmacology, for its cytotoxic, antibiotic, antifungal, insecticide activities, etc. In addition, it is reported that figs have been conventionally used for their therapeutic benefits as anticancer remedies (Guarrera, 2005; Rubnov *et al.*, 2001) [89, 90]. This traditional use of *F. umbellata* suggests that it could have anti-inflammatory properties. Nevertheless, there is no scientific report on anti-inflammatory effects and phytochemical composition of *Ficus umbellate.*

Fig 1: *F. umbellata* collected from from Chimaroke road, Agbani Nkanu West Local Government Area of Enugu State Nigeria.

Materials and Methods

Materials

Solvents (Methanol, Ethylacetate, Butanol, n-Hexane) from JHD China, Ferric chloride, Mayer's reagent, Hager's reagent, 2% hydrochloric acid, chloroform, concentrated H_2SO_4, acetic anhydride, dilute HCL, 5% $FeCl_3$, lead acetate solution, KOH solution in ethanol, 10% NH_3, Distilled water, Chloroform, Sodium hydroxide, EDTA, 2M $CaCl_2$, pure sample of aspirin.

Shed dried material of *Ficus umbellata*, Grounded material of the shed dried material, extraction bottles, measuring cylindes, weighing balance, 100ml beaker, separating funnel, filter paper, rotary evaporator, Conical flasks, Muslin cloth, filter paper (Whatman No 1), Hand gloves, Measuring cylinder. Electronic weighing balance, Test tubes, Beakers, spatula, water bath, Refrigerator, Spectronic 21D (Miltos Roy) spectrophotometer, Centrifuge.

Methods

Collection and identification of *Ficus umbellata* leaves

The leaves of a mature *Ficus umbellata* plant were collected between 8am and 11am on 10th August, 2022 from Chimaroke road, Agbani Nkanu West Local Government Area of Enugu State. The plant was identified and authenticated by Mr. Felix department of botany, International Centre for Ethnomedicine and Drug development (INTERCEDD), University of Nigeria Nsukka, Enugu state and the Voucher Number is INTERCEDD/062.

Preparation of *Ficus umbellata* leaves

The leaves of *Ficus umbellata* were washed. They were shade dried under room temperature for 28 days. The dried materials were pulverized into a coarse powder using a mechanical grinder. The powdered materials were stored at room temperature (25-27 °C) in a tightly closed container, protected from sunlight until required for extraction and analysis.

Extraction

The powdered aerial parts of 360 grams of *Ficus umbellata* was extracted for 72 hours by cold maceration technique in 2 liters of methanol in a tightly stoppered solvent bottle. The extraction was accompanied by intermittent vigorous agitation at regular intervals. After 72 hours, the mixture of powdered aerial parts and methanol were filtered through a muslin cloth and then through a separating funnel using a filter paper respectively. The filtrates were collected in conical flasks then poured into several beakers to concentrate at room temperature. The methanol extract (ME) of the leaves of *Ficus umbellata* was obtained and then stored in the refrigerator at 4 °C.

Fractionation (liquid-liquid fractionation)

This was carried out using a separating funnel. Fractionation was done in order of increasing polarity (n-hexane, ethyl acetate, and butanol) by mixing equal volume of the fractionation reagents (n-hexane, ethyl acetate, or butanol).

The dried methanol fraction, was mixed with 150ml of distilled water and macerated with n-hexane in parts using 150 ml each, seven times. The n-hexane soluble fraction was filtered and evaporated on water bath at 40° C to afford n-hexane soluble fraction. The n-hexane insoluble fraction

was then macerated with ethyl acetate in parts using 150 ml each, seven times to obtain the ethyl acetate soluble fraction. It was then filtered and evaporated to dryness on water bath at 40 ˚C. The ethyl acetate insoluble fraction was macerated seven times in parts using 150 ml of butanol each. The butanol soluble fraction was also evaporated to dryness on water bath at 40 °C. The extracts and the fractions were stored in an extract/fraction bottles.

Phytochemical screening of aerial parts of *Ficus umbellata* extract

The freshly prepared crude methanol extract was qualitatively assessed for the presence of major phytochemical constituents such as alkaloids, phytosteroids, phenolic compounds, polyphenols, tannins, phlobatanins, glycosides, quinones, anthraquinones, steriods, terpenoids, triterpenoids, saponins, flavonoids, using standard analytical phytochemical screening procedures as described by (Phytochemical methods: A guide to modern techniques of plant analysis. Springer Science & Business Media. The procedures were adopted for identification of the pytoconstituents.

Acute Toxicity Studies

In toxicity assessment of chemicals, there is no doubt that the best test species for humans are humans since accurate extrapolation of animal data directly to human may not be guaranteed due to interspecies variations in anatomy, physiology and biochemistry. However, due to ethical reasons, such chemicals are to be tested using animal models before they are subjected to human trials in humans. The conventional acute toxicity test which involves use of large numbers of animals is being replaced by alternative methods. The methods require that fewer numbers of animals or other models that do not require the use of animals (such as in silico and *in vitro* approaches) are employed (Erhirhie, Ihekwereme & Ilodigwe, 2018) [19].

Acute toxicity (LD_{50}) test

The acute toxicity of the extract was evaluated using Lorkes method with some modifications.

In the first phase, nine animals are divided into three groups of three animals each and were administered 10, 100 and 1000 mg/kg body weight of the test substance in order to establish the dose range producing any toxic effect. The number of deaths in each group is recorded after 24 hours. In the second phase, four doses of the test substance are selected based on the result of phase 1and are administered to four (4) groups of one animal each. After twenty-four hours, the highest number of death is recorded and the LD_{50} is calculated as the geometric mean of the highest non-lethal dose (a) and the least toxic dose (b). $LD_{50}= \sqrt{a \times b}$ (Erhirhie *et al.*, 2018) [19].

***In vitro* anti-inflammatory activity evaluation**

Antiplatelet aggregatory activity

About 5ml of fresh blood sample were drawn intravenously using 5ml plastic syringe into plastic tube containing 0.1ml EDTA of 1% EDTA as an anticoagulant. The tubes were centrifuged at 300rpm for 10mins and the supernatant was collected, diluted twice with normal saline and then used as the platelet rich plasma (PRP). Changes in absorption of the platelet rich plasma was determined. PRP (0.2 ml, 0.4 ml of 2M $CaCl_2$, varying concentration of normal saline and extract/fraction (s) were incubated. The absorbance of the solutions were measured at 520 nm. Changes in absorption at 520 nm were taken at intervals of 2 mins for 8 mins.

Percentage inhibition of hemolysis = (1- OD of test sample/ OD of control) x 100

Where

OD= absorbance of test sample/control

Stabilization of Human Red Blood Cell Membrane Test

Samples of the extract and fraction(s) were dissolved in distilled water/hypotonic solution. 5ml of the hypotonic solution containing graded doses of the extract and fraction (100, 200, 400, 600, 800µg/ml) were put in duplicate pairs (per dose) of the centrifuge tubes. Control tubes contained 5ml of the vehicle (distilled water) and 5ml of 200µg/ml of Aspirin respectively. Erythrocyte suspension (0.1ml) was added to each tube and moved gently. The mixtures were incubated for 1hr at room temperature (37 °C) and afterwards centrifuged for 3minsat 1300g. Absorbance (OD) of the hemoglobin content of the supernatant was estimated at 540nm using spectronic 21D (Miltos Roy) spectrophotometer. The percentage hemolysis was calculated.

Determination of percentage inhibition of haemolysis

$$\% \text{ Inhibition of haemolysis} = 1- \left\{ \frac{OD_2 - OD_1}{OD_3 - OD_1} \right\} * 100$$

Where

OD_1= absorbance of test sample in isotonic solution

OD_2= absorbance of test sample in hypotonic solution

OD_3= absorbance of control in hypotonic solution

Results

Yield of *Ficus umbellata* leaves after extraction

The initial mass of *F. umbellata* was 360 g while the mass of final extract was 29.35 g. The percentage yield was calculated to be 8.15% as seen in Table 3.1

Table 1: Yield of *F. umbellata* aerial parts

Mass of initial powder (M1) in gram	Mass of final extract (M2) in gram	Percentage yield (%)=M2/M1 × 100
360	29.35	8.15

Phytochemicals present in methanol extract of the leaves of *Ficus umbellata*

The result of the phytochemical screening shown in Table 3.2 revealed that methanol extract of aerial part of *F. umbellata* contained alkaloids, phytosteroids, phenolic compounds, terpenoids, flavonoids, tannins, saponins, steroids, quinones, and triterpenoids.

Table 2: Phytoconstituents of methanol extract of aerial part of *F. umbellata*

S/N	Phytoconstituents	Results
1	Alkaloids	+
2	Tannins	+
3	Saponins	+
4	Steroids	+
5	Terpenoids	+
6	Phenolic compounds	+
7	Quinones	+
8	Flavonoids	+
9	Glycoside	-
10	Phytosteroids	+
11	Triterpenoids	+
12	Phlobatanins	-
13	Anthraquinones	-
14	Polyphenols	-

***Source*:** Experimental results
+ indicated positive, - indicated negative.

Table 3: Acute toxicity studies Part 1

Dose	Number of animals	Observation for methanol extract of leaves	Interpretation
10 mg/kg	3	0/3	No death
100 mg/kg	3	0/3	No death
100 mg/kg	3	0/3	No death

Part 2

Dose	Number of animals	Observation for methanol extract of leaves	Interpretation
1600 mg/kg	1	0/1	No death
2900 mg/kg	1	0/1	No death
5000 mg/kg	1	0/1	No death

Antiplatelet aggregatory activity

Table 3.1: Effect of Methanol extract (crude), n-hexane, ethyl acetate, butanol fractions of *F. umbellate* leaves and aspirin on Calcium Chloride-Induced Platelet Aggregation.

	Concentrations (µg/ml)	Δ Absorbance (520nm) 30 seconds	Δ Absorbance (520nm) 60 seconds	Δ Absorbance (520nm) 90 seconds	Δ Absorbance (520nm) 120 seconds
Crude Extract	50	0.715±0.979^{abc}	0.480±0.667^{abcd}	0.244±0.334^{a}	0.126±0.161^{ab}
	100	0.121±0.171^{abc}	0.370±0.510^{abcd}	0.615±0.867^{a}	0.126±0.172^{ab}
	200	-0.184±0.253^{abc}	0.002±0.002^{abcd}	0.092±0.125^{a}	0.184±0.254^{ab}
	400	-0.600±0.818^{abc}	-0.367±0.509^{abc}	0.000±0.000^{a}	0.074±0.100^{ab}
	800	-1.176±1.629^{abc}	-1.035±1.446^{a}	-0.429±0.59 ^{a}	-0.159±0.219^{a}
Hexane Fraction	50	-3.410±5.135^{a}	0.993±0.475^{abcd}	-0.004±0.629^{a}	0.557±0.160^{ab}
	100	0.069±0.624^{abc}	0.356±0.020^{abcd}	0.787±0.586^{a}	-0.080±0.376^{a}
	200	-0.279±0.030^{abc}	0.482±0.044^{abcd}	0.409±0.152^{a}	0.281±0.030^{ab}
	400	-0.981±0.324^{abc}	-0.292±0.038^{abc}	0.053±0.074^{a}	-0.066±0.093^{a}
	800	-1.600±0.369^{ab}	-0.699±0.241^{ab}	-0.435±0.127^{a}	-0.216±0.18^{a}
Ethyl acetate Fraction	50	1.096±1.549^{abc}	0.854±0.058^{abcd}	0.273±0.386^{a}	0.137±0.193^{ab}
	100	0.236±0.005^{abc}	0.590±0.153^{abcd}	0.480±0.347^{a}	0.605±0.523^{ab}
	200	0.447±0.285^{abc}	0.463±0.039^{abcd}	0.109±0.154^{a}	0.342±0.135^{ab}
	400	0.353±0.228^{abc}	0.893±0.044^{abcd}	0.300±0.671^{a}	0.282±0.152^{ab}
	800	-0.041±0.591^{abc}	0.276±0.605^{abcd}	-0.192±0.272^{a}	0.00±0.00^{a}
Butanol Fraction	50	0.567±0.200^{abc}	1.092±0.871^{bcd}	0.673±0.276^{a}	0.799±0.110^{ab}
	100	0.128±0.181^{abc}	0.746±0.398^{abcd}	0.259±0.366^{a}	0.494±0.038^{ab}
	200	0.770±0.391^{abc}	0.768±0.034^{abcd}	0.516±0.023^{a}	0.519±0.024^{ab}
	400	1.036±0.334^{abc}	0.752±0.304^{abcd}	0.595±0.078^{a}	0.463±0.271^{ab}
	800	0.627±0.183^{abc}	1.387±0.163^{bcd}	0.388±0.908^{a}	0.640±0.170^{ab}
Aqueous Fraction	50	2.105±1.182^{bc}	0.914±0.070^{abcd}	0.761±0.158^{a}	0.987±0.472^{ab}
	100	0.851±0.354^{abc}	1.161±0.065^{bcd}	0.681±0.098^{a}	0.342±0.050^{ab}
	200	0.767±0.682^{abc}	1.063±0.284^{abcd}	0.610±0.043^{a}	0.618±0.050^{ab}
	400	0.890±0.135^{abc}	1.065±0.371^{abcd}	0.606±0.094^{a}	0.609±0.502^{ab}
	800	0.247±1.573^{abc}	0.896±0.357^{abcd}	0.788±0.200^{a}	0.560±0.129^{ab}
Aspirin	50	3.905±0.350^{c}	1.054±1.491^{abcd}	0.813±0.678^{a}	0.822±0.690^{ab}
	100	0.318±0.835^{abc}	1.004±0.123^{abcd}	0.584±0.045^{a}	0.571±0.369^{ab}
	200	1.822±0.368^{abc}	1.644±0.081^{cde}	1.132±0.234^{a}	1.421±1.089^{ab}
	400	3.668±2.767^{bc}	2.071±0.588^{de}	1.688±0.944^{ab}	1.765±1.403^{b}
	800	3.506±2.087^{bc}	3.685±0.084^{e}	3.409±1.78^{b}	0.753±0.487^{ab}

Data expressed as mean ± SD. Data in the same column with the same superscripts are not significantly different according to one way ANOVA followed by Tukey Post-Hoc test at $p< 0.05$ significant difference using n = 2

Hypotonic induced haemolysis

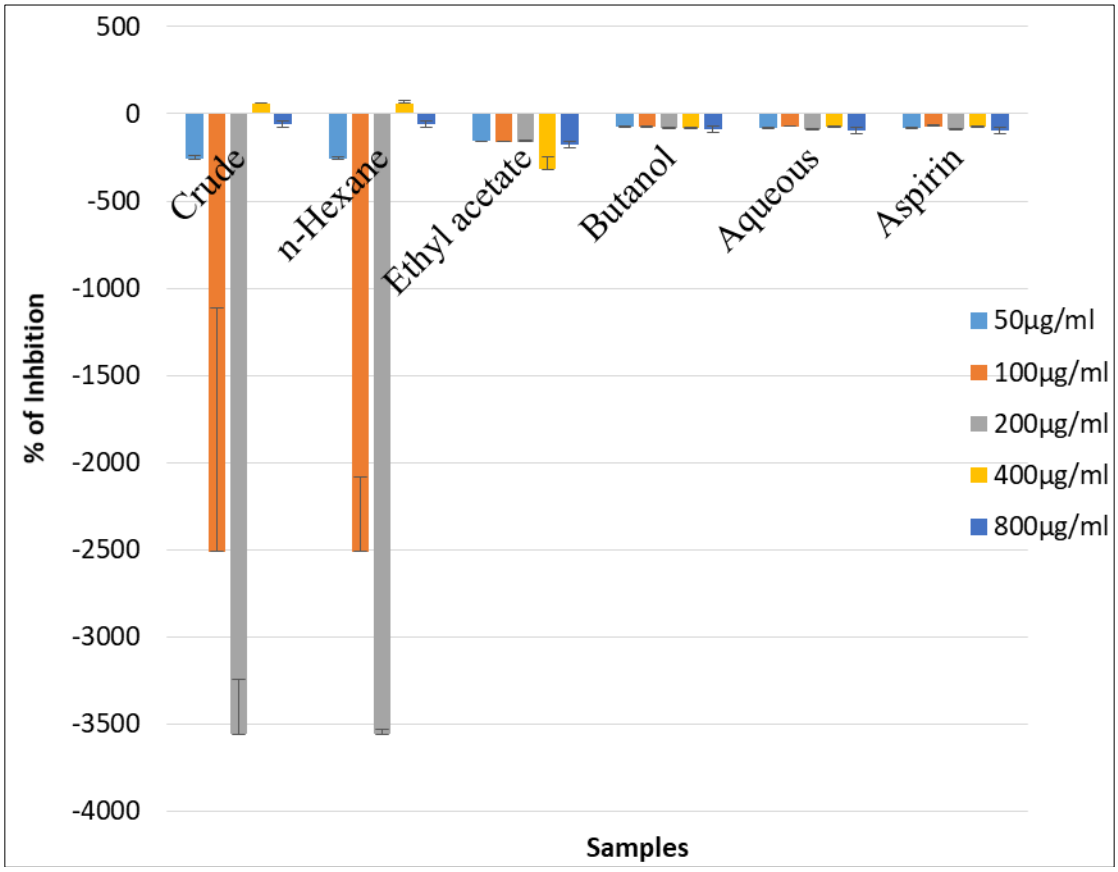

Fig 1: Effect of Methanol extract (crude), n-hexane, ethylacetate, butanol fractions of *F. umbellate* leaves and aspirin on hypotonic solution-induced hemolysis of erythrocyte membrane. *Each value* is expressed as mean ± SD (n = 2).

Discussions

Ficus umbellata is a plant belonging to the Moraceae family and is native to the tropical regions of Africa. The result of the acute toxicity studies showed no obvious toxicity or death at 5000 mg/kg showing that the LD_{50} of the leaves of *Ficus umbellata* is greater than 5000 mg/kg. This study aimed to evaluate the phytochemical constituents and anti-inflammatory activity of the aerial part of *Ficus umbellata* plant using *In vitro* antiplatelet aggregation activity and stabilization of human red blood cell (HRBC) membrane test.

Phytochemicals observed according to the findings of this study revealed the presence of alkaloids, tannins, saponins, steroids, terpenoids, phenolic compounds, quinones, flavonoids, glycoside, phytosteroids, triterpenoids, phlobatanins, anthraquinones, and polyphenols. Previous studies have shown that *Ficus umbellata* contains several medicinal compounds, including phenolic compounds, saponins, flavonoids, alkaloids, and tannins (Verma *et al.*, 2015; Ghosh & Sengupta, 2012; Nair & Nair, 2012; Mohammadi *et al.*, 2015; Thakur & Josh, 2016) [75, 25, 51, 48, 70]. The phenolic compounds have been found to have antioxidant (Mohammadi *et al.*, 2015; Thakur & Josh, 2016) [48, 70], anti-inflammatory (Verma *et al.*, 2015; Nair & Nair, 2012; Ghosh & Sengupta, 2012) [75, 51, 25], and antimicrobial (Thakur & Josh, 2016; Ghosh & Sengupta, 2012) [70, 25] properties, while the flavonoids have been associated with cardioprotective, antidiabetic and antithrombotic activities (Thakur & Josh, 2016) [70]. The alkaloids possess antimalarial and antispasmodic activities (Nair & Nair, 2012) [51], while the saponins and tannins have been found to exhibit antidiabetic and antidiarrhoeal properties, respectively (Ghosh & Sengupta, 2012; Mohammadi *et al.*, 2015) [25, 48]. The findings of this study are also parallel to a study conducted by Jain *et al.* (2018) [92] who reported the presence of alkaloids, flavonoids, and phenolic compounds in the aerial parts of *Ficus umbellata* (Jain *et al.*, 2018) [92]. The study reported the presence of various alkaloids, including quinazoline alkaloids, quinoline alkaloids, and isoquinoline alkaloids. The study also identified several flavonoids such as luteolin, quercetin, and kaempferol, as well as phenolic compounds such as gallic acid and ellagic acid. Another separate study conducted by Sharma *et al.*, (2019) [91] that examined the phytochemical constituents of the aerial parts of *Ficus umbellata* reported the presence of saponins, alkaloids, tannins, flavonoids, and phenolic compounds. This study however explicitly mentioned that they observed alkaloids in the aerial parts of *Ficus umbellata* such as quinazoline alkaloids, quinoline alkaloids, and isoquinoline alkaloids, and also, the flavonoids identified by the researchers included luteolin, quercetin, and kaempferol (Sharma *et al.*, 2019) [91]. The phenolic compounds identified in this study included gallic acid, ellagic acid, and vanillic acid (Sharma *et al.*, 2019) [91].

Thus, the results of these studies provide further evidence that the aerial parts of *Ficus umbellata* contain a wide range

of biologically active compounds such as alkaloids, flavonoids, and phenolic compounds, among others, which may be useful for various medicinal applications corroborating the findings of this paper.

In addition to the findings on the phytoconstituents, the study also evaluated the antiplatelet aggregation activity of *Ficus umbellata in vitro*. The results of this study revealed that after testing the hexane, butanol, aqueous, ethyl acetate, and crude fractions for their antiplatelet aggregation activity in comparison to the Aspirin sample, the hexane, ethyl acetate, crude extract, and butanol fractions exhibited significant antiplatelet aggregation activity at low concentrations for the two doses. This indicates that the aerial part of *Ficus umbellata* contains compounds that possess cardioprotective and antithrombotic activities. A study conducted by Kumar *et al.* (2015) [34] similarly showed that *Ficus umbellata* has potent antiplatelet aggregation activity when compared to the positive control, aspirin. The study showed that *Ficus umbellata* was able to inhibit the aggregation of platelets in a dose-dependent manner. Additionally, the study also showed that *Ficus umbellata* extracts had a greater inhibitory effect on platelet aggregation compared to aspirin. Another parallel study conducted by Dhanabalan *et al.,* (2010) [17] showed that *Ficus umbellata* had an inhibitory effect on platelet aggregation in a dose-dependent manner. However, the study found that *Ficus umbellata* was not as effective as aspirin in inhibiting platelet aggregation. These findings are also supported by a study conducted by Raut *et al.,* (2012) [59]. This study showed that *Ficus umbellata* had an inhibitory effect on platelet aggregation in a dose-dependent manner. However, the study also found that *Ficus umbellata* had a lower inhibitory effect on platelet aggregation compared to aspirin.

The stabilization of human red blood cell membrane activity of *Ficus umbellata in vitro* is an important aspect of biomedical research, as it is essential to understand how certain compounds can interact with red blood cell membranes (Anusree *et al.,* 2013; Kumar *et al.,* 2008) [7, 37]. Findings from the study regarding the hypotonic induced hemolysis in table 3.3 above showed that the crude fractions of the aerial parts of *Ficus umbellata* inhibited hemolysis and instead stabilized the red blood cells. Concurrent studies have investigated the use of *Ficus umbellata* extracts to stabilize human red blood cell membranes *in vitro*. Studies have found that *Ficus umbellata* extract can stabilize red blood cell membranes *in vitro*, suggesting that the extract has a protective role against the damage caused by oxidative stress (Kumar *et al.,* 2008) [37]. Another study conducted by Anusree *et al.,* (2013) [7], concerning the ability of *Ficus umbellata* extract to stabilize red blood cell membranes *in vitro* was examined. The study found that *Ficus umbellata* extract was able to reduce the level of hemolysis, a marker of membrane damage, in red blood cells after exposure to oxidative stress. This suggests that the extract may be useful in protecting red blood cell membranes from oxidative damage. In a more recent study, Singh *et al.,* (2016) [66] found that *Ficus umbellata* extract was able to protect red blood cells from oxidative stress, suggesting that the extract may have a beneficial role in protecting red blood cells from oxidative damage. The study also found that the extract was able to increase the membrane stability of red blood cells, which may be due to the presence of antioxidants in the extract. Overall, the findings of previous studies indicate that *Ficus umbellata* extract has the potential to stabilize human red blood cell membrane activity *in vitro*, suggesting that the extract may be beneficial in protecting red blood cells from oxidative damage which corroborates the findings of this study. However, further research is needed to fully understand the mechanisms by which *Ficus umbellata* extract is able to stabilize red blood cell membranes.

Conclusions

Evidence from the results posits that the mechanism of anti-inflammatory activity of the crude and fraction of *Ficus umbellata* might be by inhibition of platelet aggregation, but not by stabilizing the red blood cells as shown by the results. Ergo, it can be seen that *Ficus umbellata* has an inhibitory effect on platelet aggregation in a dose-dependent manner. Furthermore, results showed that *Ficus umbellata* is not as effective as aspirin in inhibiting platelet aggregation. Therefore, more research is needed to determine the therapeutic potential of *Ficus umbellata* regarding antiplatelet aggregation activity.

Funding

Funded by the authors

Acknowledgments

The authors are grateful to the technical staff of Department of Pharmaceutical Chemistry, faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, for providing access to their laboratory.

Conflicts of interest

All the authors have no conflicts of interest to declare.

References

1. Adegbolagun TA, Adewuyi A. Phytochemical screening and proximate analysis of *Ficus umbellata* (Moraceae) leaves. Journal of Pharmaceutical and Biological Sciences. 2017;12(5):77-82.
2. Agostini-Costa TS, da Silva MV, Gontijo VS. Alkaloids. In Phytochemicals-Isolation, Characterization and Role in Human Health. In Tech; c2014. p. 155-170.
3. Agostini-Costa TS, Ribeiro PRV, de Oliveira TT. Natural Products: Chemical Composition, Biological Properties and Therapeutic Importance. Journal of Medicinal Plants Research. 2014;8(4):438-447.
4. Ahmed AB, Rao AS, Rao MV, Sahu G. Antidiabetic activity of *Ficus umbellata* leaves extract in streptozotocin-induced diabetic rats. Der Pharmacia Lettre. 2014;6(3):196-201.
5. Ajayi OE, Okesola MA, Akintayo CO. Anti-ulcer activity of ethanolic extract of *Ficus umbellata* stem bark in rats. African Journal of Traditional, Complementary, and Alternative Medicines. 2013;10(4):72-76.
6. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmunity Reviews. 2005;4(3):130-136.
7. Anusree U, Jayakumar R, Najeen K, Naidu P, Kumar PS. Protective role of *Ficus umbellata* extracts against oxidative stress mediated hemolysis in human red blood cells. International Journal of Clinical and Experimental Pathology. 2013;6(2):369-378.

8. Ao C, *et al*. Evaluation of antioxidant and antibacterial activities of Ficus microcarpa L. fil. extract. Food Control. 2008;19(10):940-948
9. Aswar M, *et al*., Anthelmintic activity of Ficus benghalensis. International Journal of Green Pharmacy (IJGP). 2008;2(3):170-172.
10. Babu K, Shankar SG, Rai S. Comparative pharmacognostic studies on the barks of four *Ficus species*, Turkish Journal of Botany. 2010;34(3):215-224.
11. Bala M, Prasad J, Singh S, Gupta P. *In vitro* evaluation of anti-inflammatory activity of root extracts of Dalbergiasissoo. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(3):735-738.
12. Bhandari PR, Thapa P, Pokharel S, Shrestha BG, Shrestha S. Evaluation of anti-inflammatory activity of Ficus glomerata and *Ficus umbellata* using *in vitro* human peripheral blood mononuclear cells assay. BMC Complementary and Alternative Medicine. 2017;17(1):527. doi: 10.1186/s12906-017-2039-9
13. Cronstein BN, Sunkureddi P. Mechanistic aspects of inflammation and clinical management of inflammation in acute gouty arthritis. Rheumatic diseases clinics of North America. 2019;45(1):1-9.
14. Das S, Sahu SK, Sahoo SK, Senapati S. Anticancer potential of Ficus umbellanta; c2019.
15. De Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundamental and Clinical Pharmacology. 2013;27(2):117-127. doi: 10.1111/fcp.12003
16. Deepa P, *et al*. A role of *Ficus species* in the management of diabetes mellitus: A review. Journal of Ethnopharmacology. 2018;215:210-232.
17. Dhanabalan S, Subramanian SP, Srinivasan K. Evaluation of *in vitro* anti-platelet aggregation activity of *Ficus umbellata* Linn: A traditional medicinal plant. Asian Pacific Journal of Tropical Biomedicine. 2010;1(1):20-24.
18. Doke SK Dhawale SC. Alternatives to animal testing: A review. Saudi Pharmaceutical Journal. 2015;23:223-229.
19. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: Strengths, weaknesses and regulatory acceptance. Journal of applied toxicology. 2018;38(3):476-486. https://doi.org/10.1002/jat.3550
20. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Acute toxicity studies and determination of median lethal dose. In Basic Principles and Clinical Significance of Oxidative Stress; c2018. p. 265-278.
21. Feldman M, McMahon AT, Doogan DP. Gastrointestinal safety and tolerability of nonsteroidal anti-inflammatory drugs. American Journal of Medicine. 2019;132(4):S27-S32.
22. Firestein GS. Inflammation in rheumatoid arthritis: causes, consequences, and mechanisms. Clinical and Developmental Immunology; c2019. p. 1-2. https://doi.org/10.1155/2019/5174758
23. Firestein GS, McInnes IB, *et al*. Immuno pathogenesis of rheumatoid arthritis. New England Journal of Medicine. 2017;377(12):1245-1260.
24. Ghimire K, Bastakoti RR. Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in Central Nepal. Forest Ecology and Management. 2009;257(10):2066-2072.
25. Ghosh I, Sengupta D. Phytochemical constituents of *Ficus umbellata* and their pharmacological activities. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012;3(1):454-459.
26. Gill LS. Ethnomedical Uses of Plants in Nigeria. University of Benin Press, Benin, Nigeria, ISBN-13: 9789782027207; c1992. p. 276.
27. Gregory M, *et al*. Anti-ulcer (ulcer-preventive) activity of Ficus arnottiana Miq. (Moraceae) leaf methanolic extract. American Journal of Pharmacology and Toxicology. 2009;4(3):89-93
28. Hussain T, Tan B, Yin Y, Blachier F, Tossou MCB, Rahu N. Oxidative stress and inflammation: What polyphenols can do for us? In Polyphenols in human health and disease. Academic Press; c2016. p. 357-379.
29. Jia Q, Zhang Z, Zhang H, Wu H. Anti-inflammatory and antioxidant activities of radix isatidis extract and bioactive compounds isolated from the herb. Evidence-Based Complementary and Alternative Medicine, 2012 591728.
30. Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three *Ficus species*: An overview. International Journal of Pharma and Bio Sciences. 2010;1(4):246-253.
31. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annual Review of Immunology. 2010;28:573-621.
32. Kaur R, Arora S. Alkaloids: important therapeutic secondary metabolites of plant origin. Journal of Pharmacognosy and Phytochemistry. 2015;4(2):196-201.
33. Kaur R, Arora S. Alkaloids–Important therapeutic secondary metabolites of plant origin. Journal of Critical Reviews. 2015;2(2):1-6.
34. Khedr AI, *et al*., Panduramides A-D, new ceramides from Ficus pandurata fruits. Phytochemistry Letters. 2018;23:100-105
35. Kim SH, Jun CD. Antiplatelet activities of natural products from marine organisms. Journal of Pharmacy and Pharmacology. 2014;66(1):1-16.
36. Kumar GV, Suresh B, Reddy BS. Evaluation of antiplatelet aggregation activity of *Ficus umbellata* Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(2):258-260.
37. Kumar PS, Anusree U, Naidu P, Jayakumar R. *Ficus umbellata* extract: a potent inhibitor of human red blood cell hemolysis. Indian Journal of Pharmaceutical Sciences. 2008;70(3):289-292.
38. Kumar S, Maheshwari KK, Singh V, Yadav JP. Evaluation of *in vitro* anti-inflammatory activity of crude extract and fractions of Enicostemmaaxillare (Lam.) Raynal. Asian Pacific Journal of Tropical Medicine. 2014;7:S341-S345.
39. Kunnumakkara AB, Sailo BL, Banik K, Harsha C, Prasad S, Gupta SC, *et al*. Chronic diseases, inflammation, and spices: how are they linked? Journal of Translational Medicine. 2016;14(1):1-14.
40. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2019;24(11):1287-1294.
41. Maionea F, Cui H, Fang J, Zuo Z, Junliang D. Medicinal plants with anti-inflammatory activities. Natural product research, 1-10.www.wjpr.ne. 2015;6(17):2017, 138.

42. Mandal TK, Das NS, Mukherjee S. *In vitro* studies on the stabilization of human red blood cells by natural and synthetic compounds: A comprehensive review. Journal of Advanced Pharmaceutical Technology and Research. 2013;4(1):6-14.
43. Mashhadi NS, Ghiasvand R, Askari G, Hariri M, Darvishi L, Mofid MR. Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. International journal of preventive medicine. 2013;4(1):S36-42.
44. Masoumeh F. Flavonoids and Phenolic Acids: Role and Biochemical Activity in Plants and Human. Journal of Medicinal Plants and By-product. 2015;1(1):1-10.
45. Mazimba O. Alkaloids from plants used in traditional medicine. Medicines. 2013;1(3):56-71. doi: 10.3390/medicines1030056
46. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428-435. doi:10.1038/nature07201
47. Middleton EJr, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. The flavonoids: Advances in research since. 1994;1986:619-652.
48. Mohammadi M, Khoshdel A, Zabihi A. *In vitro* antiplatelet aggregation activity of *Ficus umbellata* extract. International Journal of Pharmacognosy and Phytochemical Research. 2015;7(6):1084-1088.
49. Mousa O, *et al*. Bioactivity of certain Egyptian *Ficus species*. Planta Medica. 1992;58(S1):632-633.
50. Mulla SI, Patil SV, Jadhav RB, Patil RY. Phytochemical and antibacterial activity of leaves of *Ficus umbellata* Vahl. Journal of Applied Pharmaceutical Science. 2013;3(3):75-78.
51. Nair MG, Nair V. Therapeutic potential of *Ficus species*. Journal of Ethnopharmacology. 2012;142(2):524-533.
52. Nayak BS, Marshall JR. Phytochemical constituents and antimicrobial activities of *Ficus umbellata* (Moraceae) leaf extracts. International Journal of Current Microbiology and Applied Sciences. 2019;8(8):1181-1187.
53. Nayak BS, Dinda SC, Nayak SS. Antidiabetic activity of *Ficus umbellata* Vahl. leaves: A potential oral hypoglycemic agent. Indian Journal of Pharmacology. 2014;46(3):268-273. doi: 10.4103/0253-7613.132176
54. Obafemi TO, Akinmoladun AC. Phytochemical constituents and antioxidant activities of *Ficus umbellata* leaves. Journal of Pharmacognosy and Phytotherapy. 2018;10(9):161-166.
55. Ogbadoyi EO, Aliero AA, Bello MI. Phytochemical and proximate analysis of *Ficus umbellata* leaves. Bayero Journal of Pure and Applied Sciences. 2019;12(1):39-44.
56. Pradhan P, Joseph L, Srinivasan KK. Antimicrobial activity of Ficus glomerataRoxb. And*Ficus umbellata*Roxb. bark extracts. Journal of Pharmacy and Pharmacology. 2016;68(3):406-413. doi: 10.1111/jphp.12533
57. Rahman MM, Islam MS, Biswas M, Khaleque A, Alam MF. Phytochemical analysis and pharmacological activities of phenolic acid derivatives. Journal of Pharmacognosy and Phytochemistry. 2016;5(2):78-83.
58. Rath SN, Padhy RN. Erythrocyte membrane stabilization activity of 3,4-dihydroxyphenylalanine: An *in vitro* study. Indian Journal of Clinical Biochemistry. 2006;21(2):173-177.
59. Raut SS, Thakare VB, Kadam SS. Evaluation of antiplatelet aggregation activity of *Ficus umbellata* Linn. International Journal of Pharmaceutical Sciences and Research. 2012;3(1):211-214.
60. Sani AY, Jimoh MO, Abdulkadir AF, Ibrahim Y, Aisha I. Phytochemical screening and proximate analysis of leaves and stem bark of *Ficus umbellata*. International Journal of Pharmaceutical Sciences and Research. 2018;9(2):567-572.
61. Sareen R, Jain SK, Sharma C. Anti-platelet activity of aqueous extract of Terminaliaarjuna bark against aspirin and ADP-induced platelet aggregation. Journal of Ethnopharmacology. 2013;150(1):192-196.
62. Sharma A, Bora KS, Sharma A. Evaluation of anti-inflammatory activity of some medicinal plants against acute and chronic inflammation. Asian Pacific Journal of Tropical Disease. 2012;2(2):S979-S982.
63. Shinde S, Sharma R. Preliminary phytochemical and pharmacological studies on Ficus racemosa (Gular). The Indian Journal of Medical Research. 1969;57(6):1070-1074
64. Shukla R, *et al*. Antioxidant effect of aqueous extract of the bark of Ficus benghalensis in hypercholesterolaemic rabbits. Journal of Ethnopharmacology. 2004;92(1):47-51
65. Siddiqui MZ. Boswelliaserrata, a potential antiinflammatory agent: an overview. Indian J Pharm Sci. 2011;73(3):255-261. doi:10.4103/0250-474X.93507
66. Singh N, Anusree U, Naidu P, Jayakumar R. Protective role of *Ficus umbellata* extract against oxidative stress mediated membrane damage in human red blood cells: A study using electron spin resonance spectroscopy. Food and Chemical Toxicology. 2016;94:213-220.
67. Singh SK, Rai PK, Jaiswal D, Watal G, Singh PP. Antioxidant activity of *Ficus umbellata* Linn. Indian Journal of Clinical Biochemistry. 2011;26(3):301-307. doi: 10.1007/s12291-011-0121-7.
68. Tasneem S, Liu B, Li B, Choudhary MI, Wang W. Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. 6 research. 2019, 139(126-140).
69. Tatti PN. Evaluation of *in vitro* anti-denaturation activity of isolated compound of Butea Monosperma bark. Pharma science monitor. 2012;3(4):2314-2320.
70. Thakur RS, Joshi L. Stabilization of human red blood cell (HRBC) membrane by *Ficus umbellata* extract. International Journal of Pharmaceutical Sciences and Research. 2016;7(8):3183-3189.
71. Uddin MZ, Hassan MA, Al Mamun A, Chowdhury MAH, Alam MS. *In vitro* anti-inflammatory and membrane stabilizing activities of methanolic flower extract of Nyctanthes arbor-tristis Linn. International Journal of Pharmaceutical Sciences and Research. 2012;3(9):3185-3189.
72. Umar MI, Junliang D, Li Y, Wang X, Zhao L. *In vivo* experimental models to investigate theanti-inflammatory activity of herbal extracts (Review). Sci. Int (Lahore). 2010;22(3):199-203.
73. Ushimaru PI, Nogueira da silva MT, Claudio Di Stasi L, Barbosa L, Fernandes A. Antibacterial activity of

medicinal plant extracts. Braz. J. Microbiol. 2007;38:717-719.
74. Vahl M. Symbolae Botanicae, Volume 3. Copenhagen, Denmark: Typis Schultzianis; c1794.
75. Verma R, Akhtar MS, Singh B, Kumar S. Phytochemical screening and evaluation of anti-diabetic activity of *Ficus umbellata* L. International Journal of Pharmaceutical Sciences and Research. 2015;6(8):3183-3186.
76. Verma S. Medicinal plants with anti-inflammatory activity. The journal of Phytopharamcology. 2016;5(4):157-159.
77. Wong KK, Yang XW. Antioxidant and anti-hemolytic activities of asiatic acid, madecassic acid, and asiaticoside from Centellaasiatica. Evidence-Based Complementary and Alternative Medicine. 2010;7(1):1-7.
78. Yadav RN, Agarwala M, Chatterjee AK. A comparative study on anti-inflammatory activity of tramadol, diclofenac and piroxicam in experimental animal models. Global Journal of Pharmacology. 2011;5(2):64-70.
79. Yang CM, Cheng YW. Rutaecarpine suppresses human platelet aggregation and *in vitro* proliferation of vascular smooth muscle cells. BioMed Research International; c2014. p. 1-1.
80. Zhao L, Hu C, Zhang P, Jiang H. Stilbenes: Molecular Mechanisms of Antioxidant, Anti-Inflammatory, and Antitumor Effects. Journal of agricultural and food chemistry. 2015;63(14):3266-3278.
81. Zhou Y, Hong X, Huang X. Antioxidative and Hemolysis Protective Effects of Polysaccharides from Lepidium meyenii. Oxidative medicine and cellular longevity; c2016.
82. Zhou Y, Hong Y, Huang H. Triptolide Attenuates Inflammatory Response in Membranous Glomerulo-nephritis Rat via Downregulation of NF-κ B Signaling Pathway. Kidney Blood Press. Res. 2016;41:901-10.
83. Chen HX, Chen W, Liu X, Liu YR, Zhu SL. A review of the open charm and open bottom systems. Reports on Progress in Physics. 2017 May 2;80(7):076201.
84. Abbas AK, Lichtman AH, Pillai S. Functions and disorders of the immune system. Basic Imunology. 3rd edition. Elsevier; c2009.
85. Zhang S, Yao L, Sun A, Tay Y. Deep learning based recommender system: A survey and new perspectives. ACM computing surveys (CSUR). 2019 Feb 25;52(1):1-38.
86. Fritsch J, Abreu MT. The microbiota and the immune response: what is the chicken and what is the egg? Gastrointestinal Endoscopy Clinics. 2019 Jul 1;29(3):381-93.
87. Gill R. The affective, cultural and psychic life of post feminism: A postfeminist sensibility 10 years on. European journal of cultural studies. 2017 Dec;20(6):606-26.
88. Arbonnier M. Arbres arbustes et lianes des zones sèches d'Afrique de l'Ouest. Arbres arbustes et lianes des zones sèches d'Afrique de l'Ouest. 2009:1-00.
89. Guarrera PM. Traditional phytotherapy in Central Italy (marche, abruzzo, and latium). Fitoterapia. 2005 Jan 1;76(1):1-25.
90. Rubnov S, Kashman Y, Rabinowitz R, Schlesinger M, Mechoulam R. Suppressors of cancer cell proliferation from fig (Ficus carica) resin: isolation and structure elucidation. Journal of Natural products. 2001 Jul 27;64(7):993-6.
91. Sharma P, Mehta M, Dhanjal DS, Kaur S, Gupta G, Singh H, *et al*. Emerging trends in the novel drug delivery approaches for the treatment of lung cancer. Chemico-biological interactions. 2019 Aug 25;309:108720.
92. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nature communications. 2018 Nov 30;9(1):5114.