EVALUATION OF ANTI-ULCER AND ANTIMALARIAL PROPERTIES OF ETHENOLIC EXTRACT OF TERMINALIA SUPERBA STEM BARK

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Abstract

The aim of present research is to extract the stem bark of *Terminalia superba* stem bark using alcohol and to evaluate anti-ulcer and antimalarial activity respectively.

Ethanolic extract of *Terminalia superba* stem bark was evaluated invitro antiulcer activity by indomethacin induced ulcer model and anti-malarial activity by Schizonticidal testing method Assay.

In this model, Indomethacin an (NSAID) drug induced ulcer model, the animals given with indomethacin (50 mg/kg) a caused damage to the gastric mucosa. Animals pretreated with ethanolic extract of *Terminalia superba* of 100 and 200 mg/kg and standard drug ranitidine (50 mg/kg) has significantly protected gastric lesions induced by indomethacin. In this research work, firstly, ethanolic extract *Terminalia superba* were subjected to the susceptibility assay for *Plasmodium falciparum* to find out whether the synthesized derivatives possess anti-malarial activity or not. The results revealed that alcoholic extract *Terminalia superba* shown good activity when compared to standard.

Conclusions: The research work involves screening of ethanolic extract of *Terminalia superba* stem bark for antiulcer activity by indomethacin induced ulcer model using ranitidine as a standard. From the results it is concluded that the extract shown promising anti-ulcer actitivity when compared to standard ranitidine. The ethanolic extract were also screened for antimalarial activity using *plasmodium falciparum* susceptibility assay model using chloroquine phosphate as a standard from the results it is concluded that the extract shown potent activity when compared to standard
Chloroquine.

Keywords: *Terminalia Superba* Stem Bark, Ulcer, Malarial, Antiulcer Models, Antimalarial Models.

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1. Introduction

In Benin, herbal preparations are commonly used as a treatment of diseases. More than 80% of the West African population use traditional medicine to overcome sickness[1]. This is motivated by the frequent failure of conventional pharmaceutical treatments and high drug prices for developing countries.[2]

Traditional care administrators vary plants combination, regardless of their toxicity or their interactions [3].The pharmacokinetic and pharmacodynamic data of these products are not often known. This could eventually cause treatment failures or accidents. Several studies on traditional treatments revealed insufficient data on plants used in medicine[4]. Medicinal plants, despite their therapeutic effects, should be used with the utmost caution as they may have a high toxicity risk. Terminalia superba (T. superba) is a tree of the Combretaceae family. T. superba extracts are used for their antimicrobial anti-ulcer, analgesics and cicatrization properties. Scientific evidence for their efficacy is widely studied but systemic safety studies are lacking. [5-7]

superba bark powder in wistar r

Fig 1: *Terminalia superba* stem bark

Ulcers

Open sores on the skin lining or mucous secreting membrane characterized by a loss of upper layer of the tissue are called Ulcers. Our alimentary tract consists of the passageway, stomach and intestine. Most ulcers are settled within the small intestine (duodenal ulcers) and on abdomen (gastric ulcers). Less or sharp higher abdominal pain with burning sensation are the key symptoms of abdomen or peptic ulcer. Acid reflux or pyrosis, feeling gorged (full) bleeding, obstruction and perforation are also associated in ulceration condition.[8] In the USA, 435 000 admissions and 796 000 emergency department visits for damage to the gastrointestinal tract occurred in 2012, accounting for one-hundredth of all duct emergency admissions that year.[9]

Ulcers typically develop as a result of an upset in the normal balance brought on by either increased aggressiveness or decreased mucosal resistance to potential inflammatory substances. There are now two major methods for treating peptic ulcers: protecting the gastric mucosa against the causes of the ulcer and lowering the generation of stomach acid. If left untreated, may result in additional issues such gastrointestinal bleeding, perforation, ulcer spreading to nearby organs, and may result in cancer.[10-11]

Causes of Ulcers

Major causes of ulcers are [12-13]

- Infection of the stomach by a bacteria "Helicobacter pylori" (H. pylori)
- Chronic use of (NSAIDs) including aspirin, indomethacin, diclofenac, aceclofenac, ibuprofen, naproxen sodium, etodolac etc.
- Consumption of alcohol, smoking of cigarette and like products.
- There is a belief that, excessive consumption of coffee, colas, spicy foods and stress also causes gastritis which leads to ulcers.

Treatment for ulcers

A determination of *H. pylori* ulcers has resulted in a treatment for this subtype that

was only recently discovered, involving the destruction of the *H. pylori* bacteria or discontinuing the NSAIDs that are being taken previously. H_2-protein receptor antagonists, H^+/K^+ATPase (proton pump) inhibitors, antacids, sucralfate, and Prostaglandin (PG) analogues are antiulcer medications with demonstrated proven and still using in the treatment of acute ulcers.[14]. However, clinical evaluation of these medications has shown more adverse effects such as constipation, laxative effect in high dose, flatulence, systemic alkalosis, neuropsychiatric disorders, gynecomastia, impotence, elevation of serum creatinine.[15-16]

Table 1 details on drugs used in the treatment of hyperacidity

Drugclasses and examples	**SideEffects**
Anti-Muscarinicagents: Pirenzepine	Dry mouth, blurred vision,Tachycardia.
Antacids: Sodium bicarbonate, Calciumcarbonate,Aluminiumhydroxide.	Diarrhoea, constipationhypokalemia,alkalosis.
H_2-receptorblockers: Cimetidine, Famotidine,Ranitidine.	Headaches, diarrhoea, Renalproblems, confusion
Prostaglandins: Misoprostol	Diarrhoea,abdominalpain, vomiting and nausea,headache
Proton pumpinhibitors: Omeprazole,Esomeprazole,Pantoprazole	Pneumonia,headaches,diarrhoea,nausea,weakness

Methods used for evaluating antiulcer activity: Peptic ulcers can be produced in animal models using techniques including physiological, pharmacological or surgical treatments. However, rodents like mice or rats are used in the majority of peptic ulcer research investigations. Experiments are conducted on *in- vivo, in -vitro* and *in-silico*software models to screen or assess the antiulcer activity of various medicines and agents.[41]The methods used for evaluating antiulcer activity are.[17-22]

***In-Vivo* models:**

- Stress ulcer models
- Pylorus ligation in rats
- Histamine induced
- Ethanol induced
- Acetic acid induced
- Cystamine induced
- Indomethacin induced
- NSAID's induced
- Reserpine induced

***In-Vitro* models:**

- Artificial gastric acid Neutralization effects of preparation
- Determination of acid neutralization capacity: Fordtran's titration method
- Assessment of H^+/K^+-ATPase activity
- *H.Pylori* antimicrobial assay.

Malaria

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protists (a type of microorganism) of the genus Plasmodium. It begins with a bite from an infected female mosquito, which introduces the protists via its saliva into the circulatory system, and ultimately to the liver where they mature and reproduce. The disease causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death. Malaria is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas. The term malaria originates from Medieval Italian: mala aria — "bad air"; the disease was formerly called ague or marsh fever due to its association with swamps and marshland.

Malaria was once common in most of Europe and North America, where it is no longer endemic, though imported cases do occur. Other Plasmodium species cause infections in certain animals. Several mammals, birds and reptiles have their own

form of malaria Malaria is a mosquito-borne infectious disease of humans and other animals caused by protists (a type of microorganism) of the genus Plasmodium. It begins with a bite from an infected female mosquito, which introduces the protists via its saliva into the circulatory system, and ultimately to the liver where they mature and reproduce. The disease causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death. Malaria is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas.

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In this point of view, schizonticidal testing method was selected based on the ease of the test, less time consumption, robust, and economical. Further to support the selection of schizonticidal as the test assay for the synthesized derivatives, schizonticidal possess the highest hits in ELSEVIER citations for estimation of the anti-malarial susceptibility for the ethanolic extract of *Terminalia superba* stem bark.

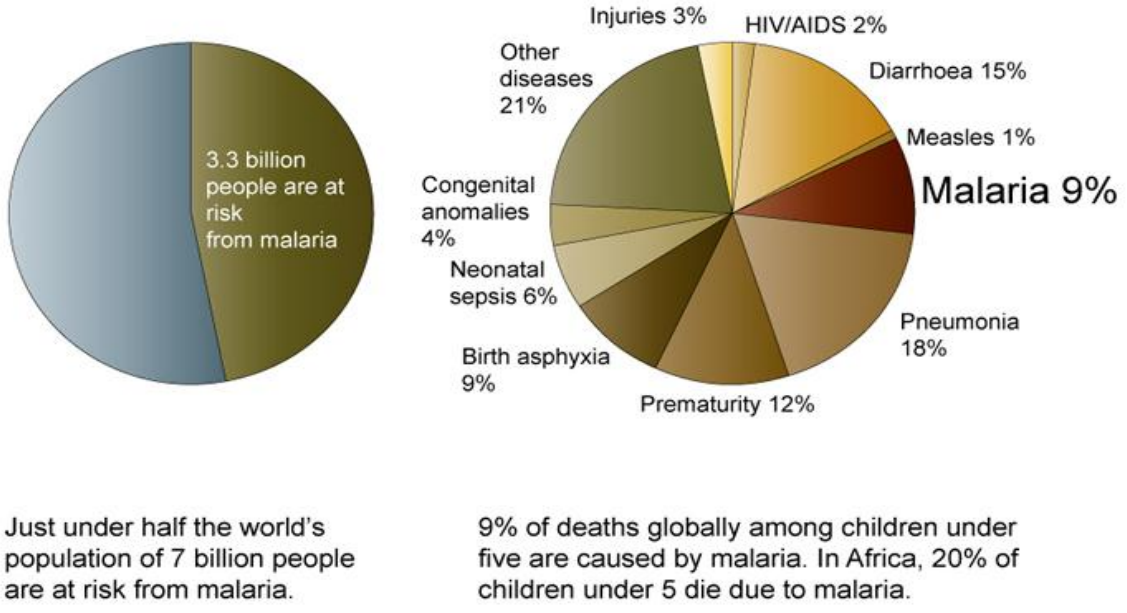


Figure 2: Diagram representation

Experimental work

***In-vivo* Indomethacin induced Anti-ulcer activity**

In this model, Indomethacin an (NSAID) drug induced ulcer model, the animals given with indomethacin (50 mg/kg) a caused damage to the gastric mucosa. Animals pretreated with of 100 and 200 mg/kg and standard drug ranitidine (50 mg/kg) has significantly protected gastric lesions induced by indomethacin**.**

Invitro antimalarial activity

Schizonticidal testing of ethanolic extract of *Terminalia superba*

- The ethanolic extract of *Terminalia superba* stem bark is dissolved in dimethylsulphoxide (DMSO) at l00mg/ml concentration. The repetitive dilutions were prepared in incomplete RPMI-1640 media (pH 7.2) up to 2mg/ml concentration.
- Add initial 100*p*l incomplete media in all eight wells of the vertical line of 96-tissue culture well plate.
- From 2mg/ml concentration 100 µI was loaded in the third well of 96-tissue culture well plate. The first well was of control containing only infected blood with medium.
- Second well was of experimental control having DMSO dilution without drug to know the level of inhibition through DMSO.
- From third to eight well proceed with double dilution method. The range of samples concentration after double dilution from 3rd to 8th well was 0.1 to 0.003 mg/well (i.e. 1 to 0.03mg/ml).
- Finally add 10(0.1 synchronized culture in each well.
- Close the plate and put in incubation for 36 hrs for the development of schizonts from rings.
- In between 36 to 40 hrs opened the plate and removed the media and prepared slides from all wells.

Derivation of IC_{50} value :

- IC_{50} value is the concentration of compound that required for inhibiting 50% schizont maturation in the parasite population.
- Count up to 200 infected RBC from each slide of eight wells.
- Calculate schizont inhibition percentage from the formula:
- 100 - [(No. of schizonts in test X 100) % No. of schizonts in control}
- Plot schizont inhibition percentage on Y-axis and $\log_2$ (logarithm of the base of the double diluted samples concentrations on X-axis.
- The shape of graph achieved was of sigmoid shape. The linear portion of the graph was used to obtain IC50 through linear regression analysis with the help of Microsoft Excel.
- The IC50 value obtained from plotted graph was actually the log of concentration based on two. Therefore it was converted first in the natural log and than, after application of anti-log it was converted to real value of sample in mg/ml.
- Data of schizont inhibition are in the form of serially increasing data therefore it was directly plotted on axis with equal interval.
- While the data of double dilution can't be plotted on the axis with the equal division therefore its log on the base of two was taken.
- The concentration of samples tested was in double dilution therefore logarithm based on two of each samples was plotted on graph.

The formula used to find log2X was log2X=log ex/log e2 (loge2=0.69).

In this method, the Inhibitory concentration (mg/ml) (IC) of the test compound can be determined by comparing dilutions of sampless with statistical significance ($P<0.001$). By using Tukey Test.

2. Results

Table 5.5: Effect Ethanolic extract of of *Terminalia superba* stem bark on ulcer index

Table 2: Effect Ethanolic extract of of *Terminalia superba* stem bark on ulcer index

Sl. No	Group	Ulcer Index
1.	Normal	0.32±0.009
2.	Ethanolic extract of of *Terminalia superba* stem bark 100mg/kg	2.48±0.04***
3.	of *Terminalia superba* stem bark Treated 200/kg	1.84±0.05***
4.	Std. Drug Ranitidine	1.16±0.03***
5.	Inducer control (Indomethacin)	6±0.17***

Results represents mean ± S.E.M., n=6. Statistical analysis was done by one-way ANOVA followed by Tukey's Multiple Comparison Test, *** p<0.0001 compared to normal

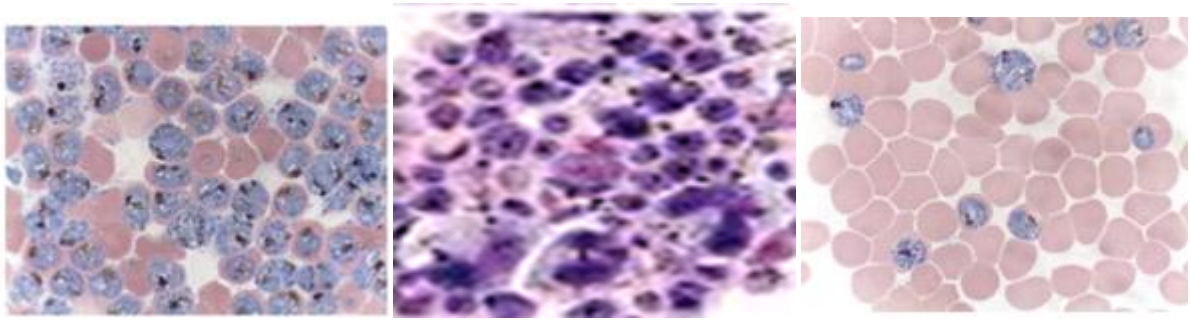
Table 3 :Effect Ethanolic extract of of *Terminalia superba* stem bark on percentage protection

Sl. No	Group	Percentage protection
1.	Normal	100%
2.	Ethanolic extract of of *Terminalia superba* stem bark 100mg/kg	56.66%
3.	of *Terminalia superba* stem bark Treated 200/kg	68.88%
4.	Std. Drug Ranitidine	81%
5.	Inducer control (Indomethacin)	--

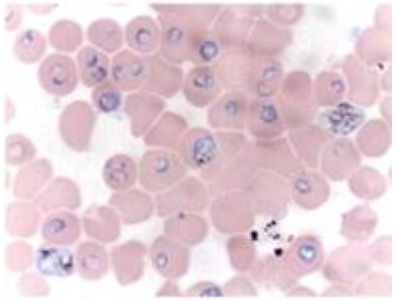
Table no.4: Inhibitory Concentration IC (mg/ml)

Compounds	Inhibition of Schizont maturation at various con. In (%) mg/ml						Inhibitory con.(mg/ml) – IC-50
	1	0.5	0.25	0.125	0.063	0.03125	
log 2x= logex/o.69	0	-1	-2	-3	-4	-5	
Ethanolic extract of *Terminalia superba* stem bark	52	46	29	16	9	2	0.9
Chloroquine Phosphate	71	24	21	19	12	8	0.5

Figure 3: Showing effect of extract of *Terminalia superba* stem bark when compared to control and standard



Culture with control *Culture with DMSO* *Culture with standard*



Culture with ethanolic extract of *Terminalia superba* stem bark

Standard Chloroquine phosphare extract of *Terminalia superba* stem bark

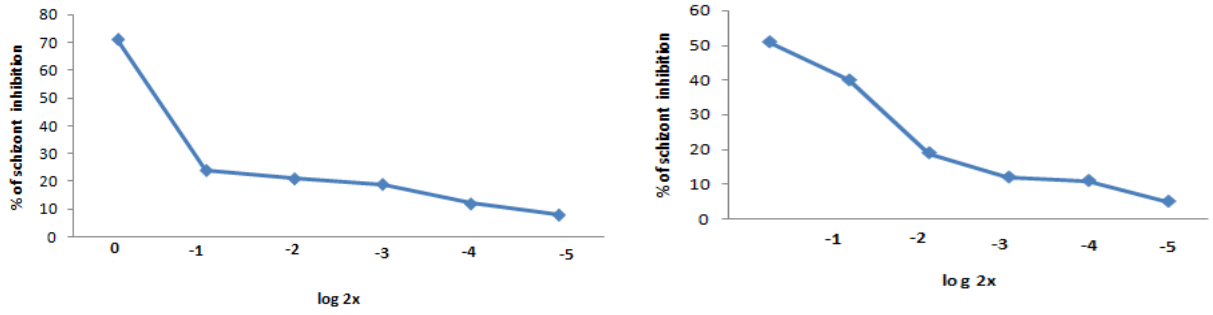


Fig 4: graph showing effect of extract on culture in comparison with standard

3. Discussion

The results anti-ulcer shows that Ethanolic extract of of *Terminalia superba* stem bark shown mild to moderate activity when compared to standard ranitidine.
The results of antimalrial of the extract possess moderate antimalarial activity when compared to standard.

4. Conclusion

In the present research work *Terminalia superba* stem bark were extracted from ethanol by successive extraction process and screened for antiulcer using indomethacin induced ulcer shown equipotent activity when compared to standard. The ethanolic extract were also screened for antimalarial activity using *plasmodium falciparum* susceptibility assay model using chloroquine phosphate as a standard from the results it is concluded that the extract shown potent activity when compared to standard
Chloroquine.

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