Antifilaria effects of *Carissa edulis* leaf extract

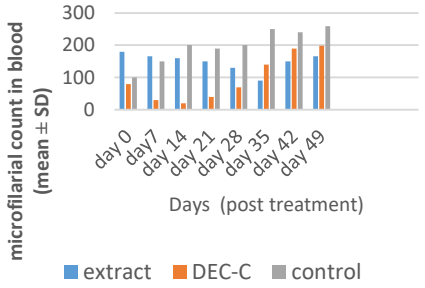
MATERIALS AND METHOD

Invivo, invitro and insilico analysis of Carissa edulis leaf extract was done using mouse model. The life cycle of *Brugia malayi* was established and maintained in mouse. *Aedes aegypti* mosquitoes was used by standard methods as a vector. The reference drug used was DEC-C (Diethylcarbamazine-citrate). HBSS was the Incubation medium used with 7.2 P.H. Sterile triple distilled water (STDW) was used to prepare DEC-C whereas Dimethyl Sulfoxide (DMSO) was used to dissolve *Carissa edulis* leaf extract. The changes in recovered worms in controlled animals over treated group was used to assess and express the efficacy of *Carissa edulis* leaf extract and DEC-C in as antimacrofilaria. Motility reduction assessment was used to assess the efficacy of *Carissa edulis* leaf and reference drug on *B. malayi* (Sanger *et al.*, 1981).. Chem3D 15.1 was used to perform the molecular docking. MetaDiscovery (Thomson Reuters, USA) database/tool was used to screen ADME/Toxicity parameters compliance. IC_{50} and CC_{50} was done.

RESULTS

Microfilarial activity: Animals treated with *Carissa edulis* leaf extract retained microfilaria below (40%) the pretreatment (0 day) level The untreated control group is progressively getting higher than the pretreatment level and never equaled the 0 day level throughout the observation period. This shows that the leaf extract of *Carissa edulis* is antifilarial. 50 mg/kg of DEC-C for 5 days caused 85% reduction in microfilarial on day 14 p.i.t. Later, it increased rapidly.By day 35 p.i.t, it crossed the pretreatment level. In controlling microfilaremia, *Carissa edulis* leaf extract was found to be good.

Microfilaricidal activity of *Carissa edulis* leaf extract and DEC-C against *Brugia malayi* in mouse model



Molecular docking of kaempferol on BmVAL-1

High binding affinity was shown in the docking results. kaempferol showed -6.1 kcal/mol affinity while reference drug, DEC showed -4.0 kcal/mol. Formation of H-bond was also shown by kaempferol (length 3.0 A˚). Stability, high binding affinity and activity of kaempferol may be as a result of the hydrogen bond. From a selection radius, from the bound kaempferol against BmVAL-1 other binding site amino acid residues were ALA 101, TYR 100, SER 122, TRP 123, VAL 99, HIS 150, PHE 84, TRP 103. These results is suggesting that the residues of amino acid of BmVAL-1 interacted well with kaempferol. According to the result of the molecular docking, it showed that there is significance similarity kaempferol had with respect to interacting amino acid residues and hydrogen bonds to that of the reference drug DEC. On molecular docking binding affinity studies, one can suggest that kaempferol is a potential antifilarial by targeting BmVAL-1.

ADME/Tox parameters evaluation

Standard descriptors and chemical parameters that was used to evaluate kaempferol and DEC showed that kaempferol was comparable to standard range and there was no predictive hepatotoxicity.

Toxicity risk assessment

The OSIRIS web server was used to evaluate the toxicity risk assessment on long term use and at high dose of DEC and the studied compound kaempferol. Four toxicity risk parameters was screened for; mutagenicity, reproductive/developmental, skin irritation, tumorogenicity, and toxicity parameters were evaluated for long term use toxicity or high doses. It showed that kaempferol has no features of risk of mutagenicity, reproductive/developmental, tumorogenicity, skin irritation and toxicity. With the above result, Kaempferol is safe for human use.

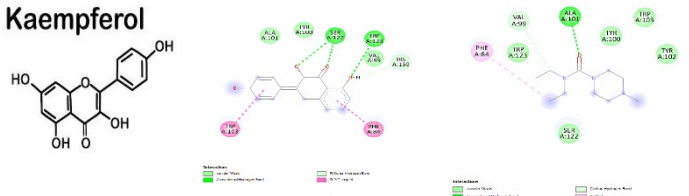
Invitro

Carissa edulis leaf extract was tested using motility assays against *B. malayi* and the results showed that *Carissa edulis* leaf extract was found to be more effective in killing *B. malayi* worms. The IC 50, LD50 and SI of leaf extract are 620 µg/mL, 895.75 µg/mL and >35 while DEC are 500, 1028 , and >22 values. Regarding normal cells, *Carissa edulis* leaf extract had a strong inhibitory effect. It showed a theoretical median lethal dose (LD50) of 895.75 mg/kg body weight for the oral route, while DEC presented an LD50 of 1028 mg/kg body weight. SI values of *Carissa edulis* leaf extract (>35) demonstrated that in vivo screening is safe to be carried out.

The motility assessment of leaf extract of *Carissa edulis*

	Loss in motility after 30 min exposure (%)	Loss in motility after 40 min exposure (%)	Loss in motility after 50 min exposure (%)	Loss in motility after 60 min exposure (%)	IC_{50} (mg/mL)	LD_{50} (mg/kg)	SI
Leaf extract	12	20	68	78	620	895.75	>35
DEC-C	18	25	77	87	500	1028	>22

The selectivity index (SI) IC_{50} (µg/mL). LD_{50}, DEC was used as a positive control (antiparasitic reference)



kaempfero l

Diethylcarbamazine (DEC)

Conclusion

In invivo treatment, the number of microfilaria reduced from 0 day throughout the post treatment observation period to day 35. The untreated control group was progressively higher than the pretreatment level and never equaled the 0 day level. DEC-treated animals' mf count dropped initially and later increased gradually over time. This shows clearly that extract was good in controlling microfilaraemia. With respect to macrofilaricidal, the finding showed that extract is clearly better in macrofilaricidal.. The in vitro result of this study demonstrated the antiparasitic effects of leaf extract evidenced by the alterations in mobility, reduction in cell viability, and changes morphology associated with cell death when exposed at different minutes. The molecular docking of kaemferol against the BmVAL-1 that was performed showed high binding affinity of kaemferol with BmVAL-1. This suggested that antifilaria effects may be explained in part by the affinity of kaemferol with BmVAL-1. The result of ADME/Tox showed that kaempferol has no features of risk of mutagenicity, reproductive/developmental, tumorogenicity, skin irritation and toxicity. With the above result, Kaempferol is safe for human use..