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Cryptosporidiosis: A Potential Anti-diarrheal Natural Product Drug Discovery Journey in Ghana, West Africa

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SKB, AGM and IA conceptualized and wrote the first draft of the manuscript. Authors SKB and IA conducted the literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

The overwhelming resistance to current drugs and the exhaustion of drug development interventions, as well as synthetic libraries, have compelled researchers to resort to the use of novel drug candidates derived from natural products. *Cryptosporidium*, the causative organism of Cryptosporidiosis, is no exception. The diarrhea-causing parasite is known to be the leading cause of deaths in children below age 5 in developing countries like Ghana and second to rotavirus as the causative agent for diarrhea in newborn calves and infants.

Currently, the only FDA approved drug for the treatment of Cryptosporidiosis is Nitazoxanide. It is, therefore, needful to develop novel alternative candidates as it could aid in the decrease in child mortality and malnutrition in developing countries. Even though there have been significant limitations into anti-cryptosporidial drug development *in vitro* and *in vivo*, essential advancements

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are being made of which this article addresses the need for research into natural products. Some studies outlined in this paper has stated potential plant extracts showing anti-cryptosporidiosis efficacy. With the wealth of medicinal plant products and *Cryptosporidium in vitro* culture expertise available in our labs at Noguchi Memorial Institute for Medical research we are certain of making potential significant strides in the world of natural product *Cryptosporidium* drug discovery in Africa.

Keywords: Drug discovery; Cryptosporidium; natural products; drug candidates; diarrhea; computational drug discovery.

1. INTRODUCTION

Discovering new effective and cheap drugs for treating diseases, both communicable and non-communicable, has been an enormous challenge in our part of the world, Africa. Even though Africa has a very rich biodiversity and hence possesses a competitive advantage over other continents, to fully develop drug candidates, even from natural sources, has not yet been possible [1]. The ultimate goal in natural products drug discovery is to deliver promising candidates which have shown sufficient evidence of biological activity at the target sites of diseases *in vitro* and *in vivo*, as well as ensure the safety of humans when introduced into the body through evaluation of drug metabolism and pharmacokinetic properties [2]. Alarming increase in resistance of most neglected tropical diseases despite efforts made by several drug development interventions and exhaustion as well as saturation of synthetic compound libraries in relation to drug production, has compelled researchers and pharmaceutical companies to gradually consider an alternative "ancestral" natural products approach. Vinblastine (*Catharanthus roseus*), Taxol (*Taxus brevifolia*) and Quinine (*Cinchona spp.*) are all natural products derivatives and are effective in treating their associated diseases [3].

Drug discovery from natural products have revolutionized medicine, over the years. The screening of microorganisms for potential antibiotic properties, stemmed from the discovery of penicillin from fungus in 1928 [4]. *Artemisia afra*, one of the most utilized plants in African ethno pharmacology is profiled to have antimalarial properties and is well known for its activity against gametocytes. Liu, Van der Kooy, & Verpoorte [5] in a review paper asserted that "*A. afra might become a future flagship species for TAM, if the problems associated with quality control could be solved and more importantly, if we can identify the active component(s), especially the antiplasmodial secondary metabolites, in this species*." These are some of the challenges associated with pursuing natural-product based drug discovery: The complexities of natural product chemistry with slowness associated in working with natural products, the intellectual property rights concerns, the challenges in ensuring access and adequate supply, the lack of appropriate well-structured guidelines for drug efficacy, toxicity, metabolism and pharmacokinetic properties are all perceived demerits of natural products drug discovery studies in Africa and beyond [6].

Almoradie et al. [7] in a review, discovered 8 different plant extracts which have shown activity against *C. parvum* and *C. hominis*, the two main *Cryptosporidium spp* that affects humans.

1.1 *Cryptosporidium spp*

Cryptosporidium is an Apicomplexan parasite that causes respiratory and gastrointestinal illnesses (cryptosporidiosis) involving watery diarrhea (intestinal cryptosporidiosis). It ranks second to rotavirus as causative organism for diarrhea in infants which is also associated with long-term growth faltering and cognitive deficiency [8]. Also, *cryptosporidium* is known to cause diarrhea in most domestic animals, wildlife and humans as well, and therefore, poses a threat to public health [9]. Options for treatment of Cryptosporidiosis are however lean Bessoff et al. [10]. Despite the intervention of advanced diagnostic techniques and treatment, diarrhea still persists to be the leading cause of death of children below age 5, in developing countries [11].

1.2 Pathogenesis & Disease

Cryptosporidium is transmitted by the spread of oocysts in faeces, which can easily be transmitted to humans through drinking of faecal contaminated water. Its association with drinking water in the community has become an issue of public concern [12]. The life cycle of *Cryptosporidium* can be grouped into 6 phases of development [13]: Excystation, merogony,

gametogony, fertilization, wall formation of oocysts and sporogony. Major parts of its life cycle reside within epithelial cells or apical surfaces, mostly in the small intestines of their hosts. Their localization in apical surfaces of hosts presents potential drug design limitations, as drugs administered orally might be effective and have local activity in the intestine without having to be absorbed extensively within mucosal layers [14].

Cryptosporidium infections manifest clinically after about 2-14 days of incubation period, which very often involves watery diarrhea, nausea, weight loss and abdominal cramps [15]. The illness is more prolonged in immunocompromised individuals than immunocompetent hosts, lasting for more than 3 weeks. The severity of the disease may differ from one individual to the other, depending on their degree of immune suppression.

1.3 Current Treatment Options and Research Situation

Previous screenings of available drug candidates showed little or no activity against *Cryptosporidium sp*. in past studies. A few drugs like Spiramycin, Azithromycin, and Bovine anti-*cryptosporidium* immunoglobulin, which were reported formerly to have some level of activity, have proven futile in clinical trials [16].

Currently, Nitazoxanide is the only FDA approved drug for the treatment of Cryptosporidiosis. A report from Amadi et al. [17], J.-F. A. Rossignol, Ayoub, & Ayers, [18] and J. F. Rossignol, Kabil, El–Gohary, & Younis [19] which involved placebo-controlled treatment trials of Cryptosporidiosis on non-AIDS patients with nitazoxanide, revealed that parasite clearance was experienced in about 93% of patients treated with nitazoxanide, whereas 37% was observed in patients treated with placebo.

Clinical trials involving nitazoxanide has shown positive effects on diarrhea and subsequently lessened mortality rates among infected individuals. Its response in malnourished children however, was recorded to be only 56% [17]. The drug unfortunately has not been reported to be effective in patients with AIDS [20]. Accordingly, Manjunatha et al. [8] also asserts that Nitazoxanide has limited efficacy in the most vulnerable patients. This calls for an urgent need for a safe and efficacious cryptosporidiosis drug, especially for this target group.

1.4 Current Assays Available for Cryptosporidiosis Natural Products Drug Development Studies Applicable to Low Income Countries

Antimicrobial drug development requires assays that mimic the phases which are essential for microbial growth. Symbionts like *Cryptosporidium*, pose a challenge as some challenges, due to the fact that they depend on the cells of their hosts partially or completely for development [14].

The use of multiple different cell lines can help in both the sexual and asexual development of *Cryptosporidium in vitro*:

I. Human ileocecal adenocarcinoma cell line HCT-8, is a prime model used as an assay for *Cryptosporidium* cultures [21]. This model involves infecting monolayers and optimizing the culture media by introducing nutritional supplements [22].
II. Castellanos Sparks, Nair, Castellanos-Gonzalez, & White, Gonzalez et al. [23], reported the implementation of primary human intestinal epithelial cells for *Cryptosporidium* cultures *in vitro*. This model allows for a rather complete analysis of the interaction between the parasite and the host compared to normal transformation of cell lines, as normal range of the intestinal epithelial cell differentiation is shown Miyamoto and Eckmann [14].

The establishment and maintenance of primary cultures are challenging and demanding technically. Even though the invention of these models for *Cryptosporidium* culture *in vitro* have proved to be indispensable, they may not be adaptable especially in most developing low-income countries.

1.4.1 Natural products with potential activities against *Cryptosporidium spp*

Plants have been known to play various roles in the prevention, management and treatment of diseases in mankind and animals. Many phytocompounds are known to have pharmaceutical potential against disease agents such as parasites. As reported in studies where various plant based products demonstrated inhibitory effects on *Cryptosporidium parvum* [24]. In other works, flavonoids and isoflavones such as quercetin and apigenin [25] and other phytocompounds such as Ginsenoside-Rh2 and

Curcurbitacin-B [26] were reported to have activities against *Cryptosporidium parvum* In experimentally infected mouse models, Sage (*Salvia officinalis*), Ginger (*Zingiber officinale*) and Ginseng (*Panax ginseng*) were reported to have significant activities against the parasite in various concentrations [27]. Perucci and other (S. Perrucci, G. Fichi, C. Buggiani, G. Rossi, & G. J. P. R. Flamini, 2006) researchers also demonstrated activities of mangiferin against the strain in neo-natal mouse models which strengthens the position of plants and their products as potential role materials that require thorough research in drug discovery.

The activity and active components of these plant extracts are summarized in Table 1 below:

Table 1. Activity of extracts on *Cryptosporidium spp*

Plant	Part	Active component	Target organism	Activity	References
Cinnamon (*Cinnamomum zeylanicum*)	Bark	Phenolic componds	*C. parvum*	Significantly reduces oocysts count of *C. parvum*.	1. [28] 2. [29]
Blueberry (*Vaccinium myrtillus*)	Fruit	Polyphenolic compounds	*C. parvum*	Increases the spontaneous excystation leading to the reduction of *C. parvum* oocysts.	1. [30]
Garlic (*Allium saivum*)	Bulb	Allicin	*Cryptosporidium spp*	Reduces the number of the Cryptosporidial oocysts and disrupts the normal physiological functions of the parasite.	1. [31] 2. [32]
Mango (*Mangifera indica*)	Leaves	Mangiferon	*C. parvum*	Exhibits a high percentage of *C. parvum* colonies	1. [33] 2. [34,35]
Olive Pomace (*Oleo europoea*)	Leaves	Oleuropein	*C. parvum*	Reduces *C. parvum* colonization	1. [36] Khuter et al. 2017
Onion (*Allium cepa*)	Bulb	Flavonoids and Sulphoid compounds	*C. parvum*	Induces a significant reduction in oocysts count of *C. parvum*	1. [28]
Pomegranate (*Punica granatum*)	Peel	Polyphenoids and Tannins	*C. parvum*	Eliminates shedding by oocysts and reduces C. parvum lymphatic and trophozoites infiltration	1. [37]
Oregano (*Origanum vulgare*)	Leaves	Carvacrol	*C. parvum*	Blocks the growth and development of *C. parvum*	1. [38]

1.5 Potential Drug Agents Used So Far against Cryptosporidiosis

Drug development tends to either focus on a whole-cell activity (activity-centered approach) or a specific molecular target approach. Once activity is identified, drug candidates then move from *in vitro* assays to *in vivo* studies [14].

Parasites are dependent on metabolic pathways for surviving, making these pathways potential targets for drugs. One of the most explored pathways for drug development for *Cryptosporidium* is the salvaging of host purines for the synthesis of nucleic acids. This process involves the action of inosine-5'-monophosphate dehyhrogenase, IMPDH [39]. IMPDH acts as an enzyme for inosine-5'-monophosphate conversion into xanthosine-5'-monophosphate for the biosynthesis of guanine nucleotide. Researchers have explored this salvaging activity, causing generic inhibitors of IMPDH to have significant anti-*cryptosporidial* activity [39].

Molecular targets have also given way for multiple leads on drug development for *Cryptosporidium*. For example, the inhibition of the fatty acyl-coenzyme A synthetases with triascsin C in *C. parvum*, was recorded to show activity against the growth of the parasite *in vitro* [40]. Another example is the inhibition of the calcium-dependent protein kinase 1 (CDPK1), which catalyses calcium-mediated signaling of the parasite. CDPK1 was inhibited by pyrazolopyrimidine derivative, according to Murphy et al. [41].

1.6 Gaps in Drug Development

Rifabutin and Rifamycin which have been reported to have anti-cryptosporidium activity, showed 25% decrease in *C. parvum* infection after *in vitro* tests. In a combination therapy with nitazoxanide, Cryptosporidium infections reduced by 75% [42]. There is the need for advancement in the management of Cryptosporodiosis, especially in developing countries like Ghana and other areas heavily burdened with this disease.

As phrased by Sparks et al. [23], "*A pivotal step towards this goal is the identification of specific targets.*" Unsuccessful genetic manipulation of parasite gene expression and the failure to propagate *Cryptosporidium spp. in vitro*, were both captured as major setbacks for the development of alternative drugs candidates [43]. Progress have been hindered in developing potential drug candidates against *Cryptosporidium* due to the limitations of experimental tools currently in use [44]. For some human infections, animal models are substandard, therefore novel studies have incorporated the use of human cell lines in the study of some intestinal pathogens like *Cryptosporidium* [45]. The challenge however is that, human cell lines do not support the propagation of parasites, readily.

1.7 Outlook of Study

Recent studies incline to the fact that the development of antimicrobials (potential drug candidates) against *Cryptosporidium* are feasible pharmacologically and also possible biologically. This can achieved by either progressing to explore known targets or by exploring larger compound libraries to test their activities against the parasite.

In Ghana and other developing counties, gastroenteritis causes the most deaths in early childhood [46].

Cryptosporidiosis is prevalent in Ghana for two (2) main reasons. According to Adjei et al. [47] in a short communique, the first reason is that most of the potential hosts (cattle, dogs, sheep, goats, rats and mice) for the parasite transmission are in the same settlement with humans. The second being that, the sources of drinking water (tap water inclusive) may be contaminated due to inadequate standards of water treatment to remove the parasites.

Contrary to this assertion, Manjunatha et al. [48] mentions that infection is common even in developed countries that apply advanced water treatment, and that *Cryptosporidium* is the cause of 50% of disease outbreaks linked to recreational water use in the USA. This setback is known to be as a result of infection which occurs through ingestion of the spore-like oocyst stage, which shows remarkable resistance to water chlorination.

Unfortunately the only FDA-approved drug for the treatment of cryptosporidial infections in immunocompetent individuals is Nitazoxanide, which is also not fully effective [49].

Jin et al. [49] went further to delve into the discovery of novel candidate compounds from natural products, which may possess

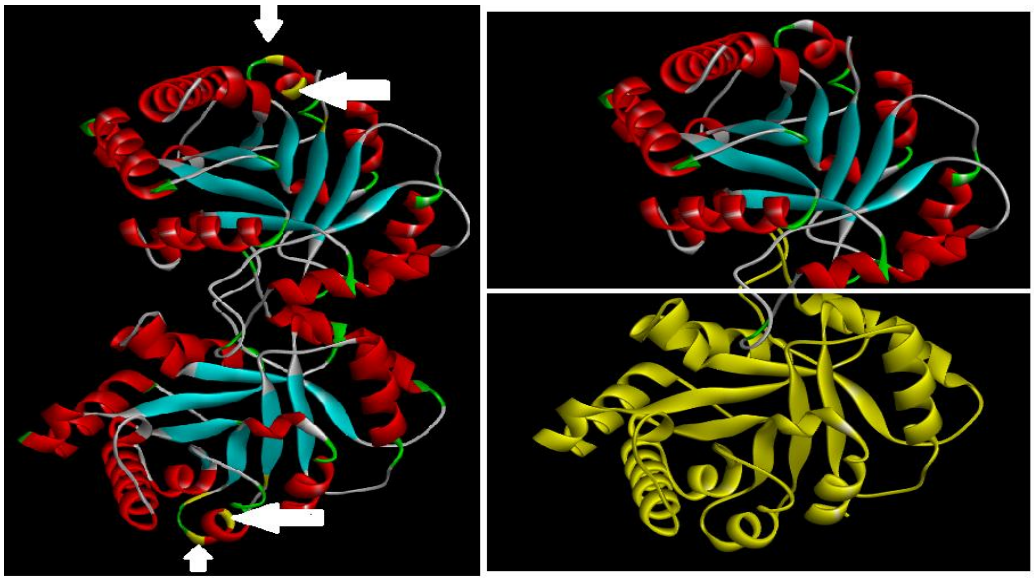

Fig. 1. 3KRS structure, an isomerase from *Cryptosporidium parvum*

anti-cryptosporidial activities *in vitro*. 800 natural products with defined chemical structures were screened phenotypically for novel activities *in vitro*, against *C. parvum*. 11 out of the 16 top hits for parasitic efficacies were plant derivates. 3 of these compounds (Cedrelone, Deoxysappanone B 7, 4'- dimethyl ether or Deox B 7, 4 and Baicalein) with submicromolar EC_{50} values had higher activities and could kill the parasite "irreversibly". It was therefore noted that Cedrelone and Baicalein were more potent than Deox B 7,4, in parasite treatment for shorter time periods. These findings provide a wide array of compounds that could be explored for anti-cryptospordial properties.

1.8 Virtual Drug Discovery Potential for Developing Countries against *Cryptosporidium*

Developing countries have done some good works particularly in the use of various plant extracts against the parasite for some time now. However, little may be achieved if the studies start and end up on extracts with little or no extensions. Despite the many challenges encountered in terms of access to state of the art technology and equipment some huge leap in research can be explored through the use of virtual technology that can be purchased or accessed on open access. Various protein-drug interactions can be stored up in natural product-linked libraries to help push for more advanced research. There is a possibility to develop capacity to interact a huge pool of phytocompounds computationally with various proteins associated with the parasite and/or the disease. Some of the interactions can be as shown in Fig. 1 a&b where 3KRS, a structure of triosephosphate isomerase from *Cryptosporidium parvum* [50] shows that it can potentially have various interaction complexes with various other active ligands from plants.

In this work, the 3KRS structure was obtained from https://www.rcsb.org/structure/3KRS and was visualized in Discovery Studio v20.1.0.19295. Fig. 1(a) shows the active sites on which various phytocompounds can be tried to dock onto and this work needs to be done on both sides of the structure as Fig. 1(b) shows it to be a dimer. This is just one simple work that demonstrate the potential that Ghana and other countries with no much equipment can pursue and contribute to research against the parasite beyond the works with extracts.

2. POTENTIAL OF ANTI-CRYPTOSPORIDIAL NATURAL PRODUCT DRUG DISCOVERY IN GHANA AND BENEFITS

The burden of disease and lack of adequate treatment regimen thereof to the disease Cryptosporidiosis caused by *Cryptosporidium* in Ghana and worldwide is still a very new area of

study especially with relation to natural product drug development research. Studies have focused intently on the importance of *Cryptosporidium* as a causative agent of diarrhea among children and its associated morbidity and mortality in Ghana and most parts of the world prevalent in the disease. Previous studies have outlined Cryptosporidiosis as a public health concern and this has led to the need of seeking for effective treatment regimens from our natural products available in Ghana which will influence our health delivery systems positively.

Currently in Africa, there has been little clinical advancement in the treatment of cryptosporidiosis and Nitazoxanide remains the only FDA-approved drug for treatment. The development of successful novel drug candidates from natural products could also aid in decreasing child mortality and malnutrition in cryptosporidioisis endemic countries including Ghana.

3. CONCLUSION

Even though there has been significant challenges and limitations in establishing robust and reliable *in vitro* and *in vivo* studies into anti-cryptosporidial drug candidates from natural products in Ghana and Africa as a whole, current efforts are being put in place to continue making essential drug discovery advancements in Cryptosporidiosis. Such efforts include various computational works in drug discovery that can help the country move a step higher in drug discovery for more meaningful research. With effective collaborations, exchange of expertise and continuous financial support are essential towards the continuous fight against Cryptosporidiosis in endemic countries such as Ghana.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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