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A Phytochemical Study of Common Plant Dietary Sources in Relevance to Inhabitants Malarial Tolerance of Kamrup Metropolitan District of Assam, India.

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Abstract

Malaria prevalence in the tropics is characterized by their typical symptoms as well as asymptomatic phase. Despite the availability of various therapies, many traditional medicines used whole plant or crude plant extracts which shows greater antiplasmodial activity. This research was carried out for determining the phytochemicals and physiochemical properties of such different varieties of plant species consumed by the community in malaria endemic and malaria tolerance zone of Kamrup Metro District, Assam. Results showed evidence of several types of plant species, which are reported to be used in the different geographical location in the world. Their extracts contain many useful phytochemicals and secondary metabolites which may contain multi-drug inhibitor or may have immunomodulatory effect as well as antiplasmodial activity. The study indicates potential inhibition factors for malaria tolerance in the community of the study area.

Keywords: *Phytochemicals, asymptomatic, antiplasmodial,immunomodulatory, tolerance.*

1. Introduction

Malaria is a fatal infectious disease which affects people of all ages in developing countries with tropical and sub-tropical climates around the world. As per World Health Organization (WHO) record reports approximately 40% of the world population lives in malaria-endemic areas, with around 300-500 million clinical cases and about 1.5-2.7 million deaths per year globally.

Human malaria is caused by five protozoan species namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P.Knowlesi* and *P. falciparum*, which has become an increasingly important clinical issue in malaria chemotherapy worldwide (Dinio *et al.*, Sashidhara *et al.*, 2012). Traditional medicines have been playing important role in the treatment of diseases including malaria since ancient time (Rudrapal, 2017). A modern chemotherapeutic form of malaria treatment started with the discovery of quinine (QN) from cinchona bark (Rudrapal, 2017). As per earlier studies, plants and/or plant-based traditional medicines are believed as the most reliable and alternative means for the discovery of new antimalarial molecules (Rudrapal, 2017). Polyphenolic flavonoids are abundant in dietary or medicinal plants, which have been identified to possess good *in vitro* and *in vivo* antimalarial activities(Al-Adhroey *et al*.,2011, Lehane *et al.*, 2008).

Plants produce many phytochemicals as secondary metabolites in their various parts. These phytochemicals play various biochemical and pharmacological role when ingested by animals (Trease, 1989). Flavonoids are the most diversified groups of phenolic compounds found in plants various parts, which impart a variety of colors such as yellow, orange, purple, blue etc. to flower petals, fruit peels, vegetables and certain grains (Rudrapal, 2017). Because of their widespread distribution in dietary plants such as fruits and vegetables, flavonoids form an integral part of human diet. Flavonoids consumed through diet as raw forms, processed products or cooked preparations contribute beneficial effects to human health (Rudrapal,2017). Flavonoids from *Artemisia annua* as antioxidants and their potential synergism with Artemisinin against malaria and cancer (Jorge *et al*., 2010).

The purpose of the study is to analyse the food diets frequently eaten in the study village where asymptomatic malaria cases were detected and to

find if there are any role in malaria tolerance with the view (1) To establish relationship common dietary ingredients with malaria tolerance and (2) To comparative quantify the phytochemicals present in the food diets in study and control villages. The result of the study will contribute towards the potential use and sources of food materials of a defined geographical location where it is cultivated and may be used as common dietary resources by the population of a defined forest area and their coexistence of malaria parasite in their blood, remaining asymptomatic in nature. It will also help health care system to help to formulate different phytochemical derivatives as medicines and can promote awareness in the community to traditional uses of food which can play role in malaria tolerance.

2. Materials and Methods

2.1 Research design and setting

The present study is based on cross-sectional research design**.** The present study has been carried out in Hazongbari sub-center under Sonapur block. The research area is selected since annual parasitic incidence is very high in the district and malaria is the major health problem in these areas due to the ecological and poor livelihood of the population. Four urban villages have been selected as a control, a total 3283 population (747 households), which are 1KM away from Panikhaity railway station and a test study area of three rural villages of 359 populations (65 households), around 10 KM away from Panikhaity railway station, Kamrup Metropolitan District, Assam. The study villages consisted of the natural reservoir of malaria transmission throughout the year. Hilly forest areas with natural pools, poor environmental sanitation with standing water bodies, foothills, paddy fields. Occupation and living habits of villagers which boasts a natural ecosystem for malaria transmission. The communities are developing resistant to malaria and mostly all are asymptomatic in nature. They are predominantly farmers mainly producing rice, grains, and vegetables consume in their diets, thus receive carbohydrate and proteins. On the other hand, the control villages are situated in plain as well as hilly areas, adjacent to hills. District administration is better and also, they are economically sound. They are basically farmers, volunteers and private sector workers. The villages are malaria endemic to the district. The households of all these villages are well trained for the all the preventive measures of malaria prevention and use of long-lasting insecticidal nets (LLIN). To evaluate the effect of intervention, fever survey and mass blood survey have been done regularly for detection and treatment of malaria.

2.2 Sample size

This study was preceded by initial visits to the study area to establish a relationship and to understand dietary habits of the communities and to seek for permissions from local leaders to conduct the research in the area. Questionnaires were given to the women group, which included about the food materials they eat and the methods of preparations. Out of 100 households, about 25 food materials, main ingredients and preparations were collected. And 10 samples were taken for phytochemical analysis which was locally cultivated and frequently used by the community of study village. Same numbers of samples were collected from the control village to define a conclusion.
Pharmacognostic and phytochemical studies were carried out in State Drug Testing Laboratory, AYUSH, Govt. Ayurvedic College and Hospital, Guwahati-14.

2.3 Extraction:

300gm of powdered was extracted successively with solvents like petroleum ether, benzene, chloroform, acetone, methanol respectively in a Soxhlet apparatus(Wallis, 1967). Each solvent extract was then concentrated by distilling off the solvent under reduced pressure.

2.4 Photochemical Screening

The aqueous and methanolic extracts along with other solvent extracts of plant materials were studied for various phytochemicals like alkaloids, flavonoids, phenols, tannins, and terpenoids by using precipitation and coloration reactions (Trease, 1996).]

2.5 Statistical Analysis

Data for the phytochemical analysis for both control and study villages were recoded for 3 independent biological replicates and were analyzed statistically by Kruskal–Wallis test with Dunn's post-test [10] to compare three or more unmatched groups using GraphPad Prism (version 5.03 for Windows; CA, U.S.A.). For all statistical analyses, significance was set to * = $0.01 \leq p \leq 0.05$, ** = $0.001 \leq p \leq 0.01$, and ***/ΔΔΔ = $p \leq 0.001$ throughout the experiments and accordingly plotted in the tables for clear understanding.

3. Result and Discussion

Herein in this study, we found out that in the study village, the common diets included locally grown plants such as, *Carica papaya, Manihot esculentum, Aka colocasia, Musa paradisiaca* L, families of Zingiberaceae, families of Poaceae, Capsicum and the many other green vegetables

like, neem, bitter guard, drumstick, other wild vegetables.

Table 1 show that there are many plants which are considered and studied as medicinal plants used for malaria therapy in Nigeria, Cameroon, and Ghana. Its use against malaria is justified by the presence of high concentrations of active compounds which has a minimal antimalarial activity and a minimal toxicity.

Table 1: Reported Plant materials used for treatment of malaria.

Sl. No	Plant materials	References
1	*Carica papaya*	Omosun G *et al.*, 2013, Betti *et al.*, 2013, Saotoing *et al*., 2011, Idowu *et al.*, 2010.
2	*Manihot esculentum*	Betti *et al*., 2013, Omosun G *et al*., 2013.
3	*Momordica charantia*	Marles and Farnsworth, 1995, Ethan *et al*., 2003. Munoz *et al*., 2000, and Inga *et al*., 2002, Singh *et al.*, 2006, Balogun *et al*., 2012.
4	*Capsicum frutescens L.*,	Mojab *et al*., 2003, Betti *et al*., 2013.
5	*Capsicum annum L*	Betti *et al*., 2013, Mojab *et al*., 2003.
6	*Curcuma longa*	Rasoanaivo *et al*., 2011.
7	*Aka colacasia*	Williams *et al*., 1981.
8	*Zingiber officinale*	Betti *et al*. 2013, Rasoanaivo *et al*., 2011, Titanji *et al*., 2008.
9	*Oryza sativa*	Betti *et al*., 2013.
10	*Colocasia esculata*	Pravakar Padhial, 2011.

The qualitative and quantities analysis (Table 2 & Table 3) showed the presence of higher amount of phytochemicals in the study. The presence of the phenolic content, total flavonoid content, alkaloid was observed most of the species. Similarly, tannin was present in *Carica papaya*, *Oryza sativa*. Terpenoid and carotenoid were present in *Capsicum frutescens*, *Capsicum annum*.

Table 2: Phytochemical analysis of plant materials of Study village.

The values in the table indicate the mean values of 3 biological replicates +/-SEM. The level of significance in indicated by *, wherever applicable.

Plant Material	Total Phenolic content (g/100g)	Total Flavonoid Content	Alkaloid	Tannin	Terpenoid	Carotenoid
Manihot esculantum	0.98 ± 0.003	0.86* ± 0.001	Present	-	-	-
Aka colacasia	22.33 ± 0.003	2.54*± 0.001	Present		-	-
Capsicum frutescens L	0.759 ± 0.003	0.306 ± 0.001	Present		Present	1.2
Capsicum annum L	0.988 ± 0.003	0.556* ± .001	Present	-	Present	1.88
Colacasia esculanta	33.33 ± 0.003	22.54 ± 0.001	Absent	-	-	-
Carica papaya	Present	-	-	Present	-	-
Zingiber officinale	-	Present	Present	-	-	-
Momordica charantia	-	Present	Present	-	-	-
Curcuma longa	-	Present	Present	-	-	-
Oryza sativa	-	Absent	Present	Present	-	-

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Table. 3: Phytochemical analysis of plant materials of Control village.

The values in the table indicate the mean values of 3 biological replicates +/-SEM. The level of significance in indicated by *, wherever applicable.

Plant Material	Total Phenolic content(g/100g)	Total Flavonoid Content	Alkaloid	Tannin	Terpenoid	Carotenoid
Manihot esculantum	0.65 ± 0.003	0.55 ± 0.001	Present	-	-	-
Aka colacasia	20.11 ± 0.003	1.99 ± 0.001	Present	-	-	-
Capsicum frutescens L.	0.754 ± 0.003	0.298 ± .001	Present	-	Present	0.6
Capsicum annum L	0.858 ± 0.003	0.458 ± .001	Present	-	Present	1.22
Colacasia esculanta	35.33 ± 0.003	23.56± .001	Absent	-	-	-
Carica papaya	Present	-	-	Present	-	-
Zingiber officinale	-	Present	Present	-	-	-
Momordica charantia	-	Present	Present	-	-	-
Curcuma longa	-	Present	Present	-	-	-
Oryza sativa	-	Absent	Present	Present	-	-

The comparative analysis of phytochemicals from food diets of study and control villages (Table 2 and 3) shows that flavonoids levels of diet constituents were significant at ($0.01 \leq p \leq 0.05$) compared to control village. This high content of flavonoids in study village diet may have contributed to anti-malarial response, which have been reported earlier (Inga *et al.,* 2002). It is also well documented that many plant secondary metabolites are increasingly synthesized under heat and drought stress, including flavonoids(Kaplan *et al.,* 2004, Wang, 2005). The Savanna monkey diets include a large quantity of flavonoid-rich leaves and bitter fruits as *Balanitesaegyptiaca.* So there are possibilities that these dietary compounds led to the reduction or elimination of simian malaria(Maranz, 2012). The human endothelial system at normal dietary plasma concentrations showed the strong effect by flavonoid in many traditional African malaria therapies and it suggests the potential for reducing the severity of malaria infections and facilitating parasite clearance via immune response(Maranz, 2010).

Moreover alkaloids, saponins, and flavonoids have been implicated to be responsible for antimalarial activity(Ettebong *et al.,* 2015) as these secondary metabolites elicit bioactivity wholly or in combination with other plants(Shigemori *et al.,*). Malaria parasites by wreaking havoc synthesize protein and produce free radicals in the human body. These vices are corrected in the presence of alkaloids which block protein-synthesis of *Plasmodium* species, and flavonoid, saponin, and tannin which are involved in primary anti-oxidation of free radicals and other reactive oxygen species(David *et al.,* 2004) In many plants, antiplasmodial activity is associated with the presence of total polyphenols, flavonoids, and alkaloids. [23] For instance, alkaloids occur in plants in association with characteristic acid(Evans, 2002) and are known to have anti-cancer, anti-aging and antiviral properties with marked physiological actions on man and animals(Ibukunoluwa,(Ibukunoluwa, 2017).

Studies show that the presence of alkaloids and terpenoids might be responsible for the antimalarial activity exhibited by *Artemisia maciverae* and *Artemisia maritime* (Ibukunoluwa, 2017). The present study shows that all the plant species daily use as diet by the community shows the presence of alkaloids and tannin (*Capsicum*) might be responsible for the antiplasmodialactivity.

The presence of higher amount of terpenoids in *Capsicum* species in the study village than the control village. Earlier research displayed various vital pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities(Mojab *et al.,* 2003).

From all the plants which are locally available and frequently consumed by the communities in the study area, which possesses an antimalarial capacity and may have indirectly provided tolerance to the malaria parasite. The survey also shows that good numbers of respondents still rely on traditional different plant species herbal remedies. The study identified 10 different plant species during the period of house visits to the

study areas and found different households usually consume either raw or cooked form. Species of *Araceae* traditionally used for malaria, fevers, headaches, and liver disorders are reviewed. [15] Relevant literature that reveals the antimalarial potential of extracts and isolated compounds including median inhibitory concentrations (IC50) against *Plasmodium falciparum*(Frausin *et al*., 2015).

4. Conclusion

This study indicates that in the study village, regular use of flavonoid-rich common dietary sources, which have the higher amount of phytochemicals than usual food sources, may have a high potential for the production of compounds for the development of anti-modulatory and antiplasmodial activity. It is reinstated in this study that the villager's diets include a large quantity of flavonoid-rich and other phytochemical-rich plant species and is comparatively higher amounts that these dietary compounds could play a role in reducing or eliminating malaria parasites possess significant suppressive effects on the human body for the developing characteristics of malaria symptoms. Further in-depth study on the efficacy of phytochemicals on malaria may lead to a new direction on research.

Conflict of Interest

No conflicting interest.

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