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Review Article

ANTIOXIDANT AGENTS ALTERNATIVE SOURCE FOR MALARIA DISEASE

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ABSTRACT

Oxidative pressure plays one of the most important role for expansion of anemia in malaria. Environment has been a source of medical agents for thousands of years and the use of medicinal plants, especially in conventional medicine, is currently well recognized and established. The junk food and environment at the present lead to the degenerative diseases to mankind. These degenerative diseases will create free radicals and resolve the major cause of degenerative diseases including malaria, while medicinal plants correspond to a wealthy source of antioxidant agents. Medicinally, these plants are used in diverse countries and are a source of many powerful and dominant drugs. The exploit of medicinal plants to treat human disease have its roots in ancient times. A broad range of medicinal plant part is used for extract as raw drugs and they possess diverse medicinal properties.

The ethanolic extracts of *Piper retrofractum, Zizyphus sativa*, *Eulophia campestris* were used in present study. The plants were screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins, glycosides etc and their effect on 2,2-Diphenyl-1-picryl-hydraxyl radical (DPPH) was used to determine their free radical scavenging activity. Consequently, in present investigations selected Indian Medicinal Plants showed appreciable antioxidant activity against radical scavenging assay. All the plants showed potent inhibition of DPPH radical scavenging activity, P.*retrofractum* the most potent. The free radical scavenging activity of these plants probably contributes to the effectiveness of the above plants in malaria therapy.

Keywords: Indian Medicinal Plants, Antioxidant Potentials, Phytochemical Screening.

INTRODUCTION

Environment disasters epidemic under way. Pollution is increasing day by day will lead to increase water pollution and many more contaminations. Therefore, malaria is becoming a global disease prevalent in the tropics caused by Plasmodium species. It is one of the oldest and greatest health challenges affecting 40% of the worlds' population. Malaria is a disease which can be transmitted to people of all ages. It is caused by parasites of the species *plasmodium* that are spread from person to person through the bites of infected mosquitoes. About 3.3 billion people - half of the world's population - are at risk of malaria. Every year, this leads to about 250 million malaria cases and nearly one million deaths. People living in the poorest countries are the most vulnerable[1]. The Indian National Malaria Eradication Program (NMEP) is reporting 2.5 to 3 million malaria cases, and about 1,000 malaria deaths yearly. Malaria in the northeastern states is steady and in the peninsular India unsteady. In attendance there are six major and three minor malaria vectors, of which Anopheles culicifacies transmits malaria in rural areas. *Plasmodium vivax* is the dominant infection and account for 60-65% cases while *P. falciparum* contributes 30-35% cases. Field operations to control malaria are impede by resistance and/or exophilic vector behavior, parasite resistance to anti-malarial drugs, operational problems in spraying, failure to search breeding of mosquitoes at weekly intervals, staff shortages and financial constraints. Resurgent malaria invaded new ecotypes created by green revolution, industrial growth and urban development resulting in paradigm shift towards man-made malaria. NMEP comprise a world bank-assisted improved malaria control project by means of primary emphasis to defend 62.2 million elevated risk populations in 7 states[2]. One of the principal reason for the expansion of anemia in malaria seems to be oxidative stress[3]. The body immune system is activated by infection counting malaria; in this way cause the discharge of reactive oxygen species [4,5,6]. In accumulation to this, the malaria parasites in addition stimulate influenced cells to create reactive oxygen species in this manner resulting in hemoglobin deprivatio[7]. Yet low intensity of plasma antioxidant has been revealed in *Plasmodium falciparum* unhygienic children moreover it has been recommended as a potential supplier to the morbidity and mortality of malaria[8].

Augmented resistances of malaria parasites to the usually used anti-malarial drugs have been reported, along with the need to strengthen research in the area of expansion of new ant malarial particularly from medicinal plants. A evaluation of the medicinal plants used in the southwestern part of Rajasthan for the healing of malaria indicate that a rich flora diversity exist in Northern hemispheres be affluent in the flora by means of ethno medicinal treasure. Consequently, the current study aimed to consider the free radical scavenging behavior of some of the frequently used medicinal plants in Mt. Abu region of Rajasthan where following plants ethanol extracts were selected extracts of the *Piper retrofractum, Zizyphus sativa*, *Eulophia campestris* Further, these plants were screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins, glycosides etc.

MATERIALS AND METHODS

Collection

Authentic samples: Various market samples of *Piper retrofractum, Zizyphus sativa*, *Eulophia campestris* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

Identification

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Processing of plant materials

During the course of the study each sample was screened for its foreign matter and milled, before use.

Experimental details

Present studies were performed on *Piper retrofractum, Zizyphus sativa*, *Eulophia campestris* for the following studies-.

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

Phytochemical Screening

Phytochemical screening was performed using standard procedure:

Test for reducing sugars (Fehlings Test)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for terpenoides (Salkowski Test)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

Test for flavonoides

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

Test for tannins

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent. The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

Antioxidant Activity

Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml (Takao *et al.,* 1994) for antioxidative (qualitative and quantitative) assay.

Qualitative assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer.

Quantitative assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (1mg/ml or 10^3µg to 10^{-3} µg/ ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

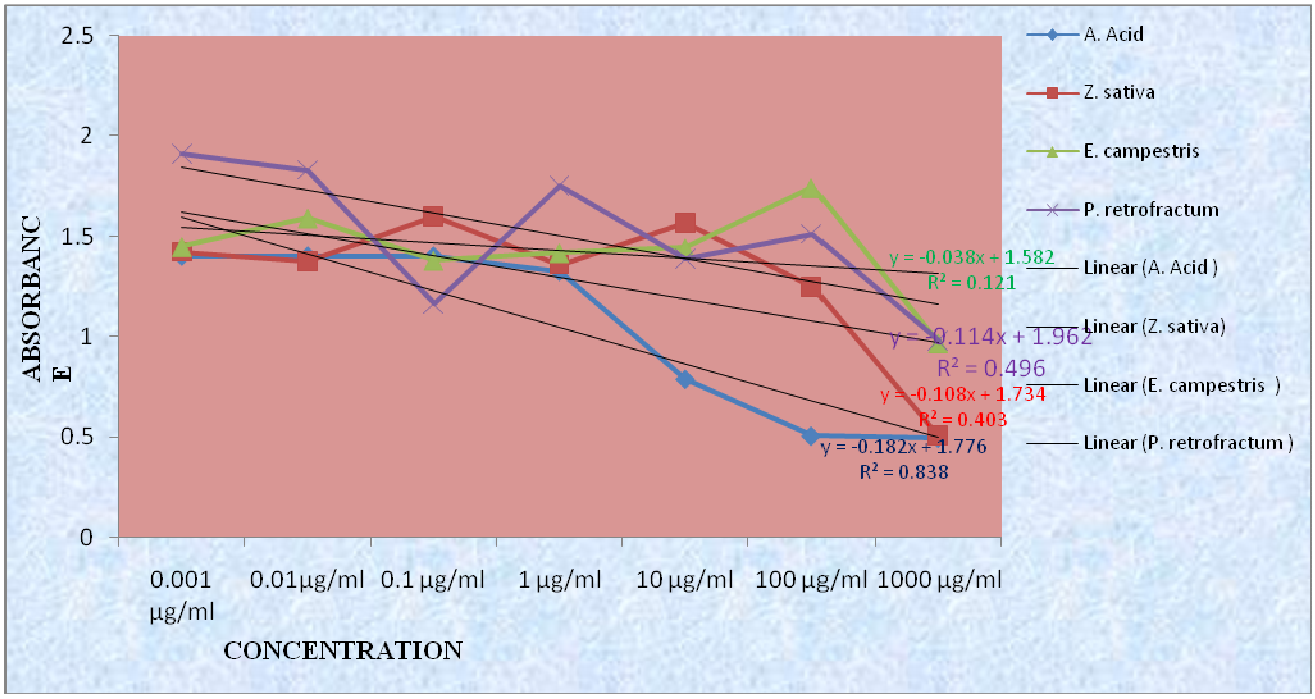

Fig. 1: DPPH Scavenging activity where concentration is plotted aginst absorbance for Indian Medciinal Plants

RESULTS AND DISCUSSION

In the present investigations antioxidant activity of *Piper retrofractum*, *Zizyphus sativa*, *Eulophia campestris* Showed good activity against the DPPH assay method where the regression line clear cut showed in figure 1, the effectiveness of it as it's have potentials which are comparable to ascorbic acid.

The photochemical screening of plants studied showed the presence of only alkaloids in CHVYA and SALAM GATTA. Only terpenoides present in CHVYA. In CHVYA presence of reducing sugar, terpenoides, flavonoides & alkaloids were observed while terpenoides were absent in UNNAB and SALAM GATTA. In UNNAB, only the presence of reducing sugar were observed while SALAM GATTA shows positive results for presence of alkaloids in Table 1.

Table 1: Phyto-chemical test of Indian Medicinal Plants

Plants	***CHVYA***	***SALAM GATTA***	***UNNAB***
Test			
Reducing sugars	+	-	+
Saponins	-	-	-
Tannins	-	-	-
Terpenoides	+	-	-
Flavonoides	+	-	-
Alkaloides	+	+	-

DISCUSSIONS

The Phytochemical screening of the plants bare a few differences in the constituent of the tested plants. Where *Piper retrofractum* possess a large amount of alkaloids, flavonoids, terpenoids and reducing sugars. The occurrence of quercetin in huge quantity is rationally proportional to the antioxidant activity so it is evidently show that occurrence of flavonoids will prove the antioxidant activity and promote a drug for treatment of malaria, so further a work on isolation of the bioactives will escort to the production of antimalarial drugs from medicinal plants. All the plants exhibited strong antioxidant activity more or less. The occurrence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids are the phenolic compounds and plant phenolics are a major group of compounds that perform as primary antioxidants or free radical scavengers. The DPPH test provides in sequence on the reactivity of the test compounds with stable free radical and it gives a strong absorption band at 517nm in visible region. Consequently, this type of studies suggests that these plants acquire antioxidant activities which can counteract the oxidative damage induced by the malaria parasite. This may be one of their modes of action in malaria therapy.

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