Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2010, 2(5): 335-340
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Phytochemical Screening and Antioxidant Activities of Some Indian Medicinal Plants Used for Malaria Therapy

Neha Tiwari[a]*, Anil Chaudhary[b], Ashutosh Mishra[c]

[a]* *Siddhartha Institute of Pharmacy, Dehradun- 248001,Uttarakhand India.*
[b] *Wochardt Ltd, Aurangabad, India.*
[c] *A.N.D College of Pharmacy, Gonda, U.P., India*

ABSTRACT

Oxidative stress has been shown to play an important role in the development of anaemia in malaria. The antioxidant activities of four medicinal plants traditionally used in the treatment of malaria were determined. The ethanolic extracts of the leaves of Vitex negundo, rhizomes of Acorus calamus, leaves of Euphorbia hirta and roots of Coleus forskohlii were used in the present study. The plants were screened for the presence of phytochemicals and, their effect on 2,2-Diphenyl-1-picryl-hydrazyl radical (DPPH) was used to determine their free radical scavenging activity. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars. The crude extract of the Euphorbia hirta, Vitex negundo, Acorus calamus and Coleus forskohlii showed maximum inhibition as 86.1% at 0.5mg/ml 78.4% at 1 mg/ml , 79.6% at 5mg/ml and71.7% at 5 mg/ml respectively which is comparable with vitamin C at the same concentrations. Vitex negundo being the most potent. The free radical scavenging (antioxidant) activities of these plants probably contribute to the effectiveness of the above plants in malaria therapy.

Key words: *V. negundo*, *A. calamus*, *E. hirta*, *C. forskohlii*, Antioxidants

---

INTRODUCTION

Malaria is 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 world's population. It affects 300-500 million people and kills 1.5-2.7 million people annually.[1] High mortality rate is reported in children and pregnant women, also the disease has a negative impact on the economy of prevalent countries.[2]

One of the major reasons for the development of anaemia in malaria seems to be oxidative stress.[3,4] The immune system of the body is activated by infections, including malaria, thereby causing the release of reactive oxygen species. In addition to this, the malaria parasite also stimulates certain cells to produce reactive oxygen species thereby resulting in haemoglobin degradation.[5,6] Indeed, depressed level of plasma antioxidants has been shown in *Plasmodium falciparum*-infected children and it has been suggested as a possible contributor to the morbidity and mortality of malaria.[1] Increased resistance of malaria parasites to the commonly used antimalarial drugs have been reported, and hence the need to intensify research in the area of development of new antimalarial drugs especially from medicinal plants.

A review of the medicinal plants used in the India for the treatment of malaria indicates that a rich flora diversity exists in India.[7] The present study aims to investigate the free radical scavenging activities of some of the commonly used medicinal plants in India. The following plants were selected for investigation: *Vitex negundo*, *Acorus calamus*, *Euphorbia hirta*, *Coleus forskohlii* . The decoctions of the leaves of *Vitex negundo*, rhizomes of *Acorus calamus*, leaves of *Euphorbia hirta* and roots of *Coleus forskohlii* are commonly used in the traditional treatment of malaria in India.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves of *Vitex negundo*, rhizomes of *Acorus calamus*, leaves of *Euphorbia hirta* and roots of *Coleus forskohlii* were collected from High Altitude Plant Physiology Research Centre (HAPPRC), Srinagar, Uttarakhand, India in the month of Mar 2010 and were taxonomically authenticated by the Dr.Alok Lehri, Scientist E-1,Central Instrumental Facility, National Botanical Research Institute, Lucknow, India. The leaves were air dried for 20 days, crushed into coarse powder with a grinder and passed through 40-mesh sieve. They were stored in a well closed container.

Extraction of plant materials

The plant materials (leaves of *Vitex negundo*, rhizomes of *Acorus calamus*, leaves of *Euphorbia hirta* and roots of *Coleus forskohlii*) were air-dried at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. The ethanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 1 L of ethanol at room temperature for 48 h. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40°C. The percentage yield of extracts ranged from 8–21%w/w.

Phytochemical screening

Phytochemical screening were perfomed using standard procedures.[8,9]

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g 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 anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test

tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test)
To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for flavonoids
Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappear on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

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

Test for tannins
About 0.5 g of the extract was boiled in 10 ml 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 colouration.

Test for alkaloids
0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)
To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Determination of antioxidant activity
The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described.[10,11] The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in methanol (Analar grade). Vitamins C was used as the antioxidant standard at concentrations of

0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/ml. I ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \{[A_b - A_a]/A_b\} \times 100 \ldots\ldots (1)$$

where A_b is the absorption of the blank sample and A_a is the absorption of the extract.

RESULTS

Phytochemical screening of plant materials

The phytochemical screening of the plants studied showed the presence of flavonoids terpenoids, saponins and tannins (Table 1), *Vitex negundo*, *Coleus forskohlii* and *Euphorbia hirta* showed the absence of anthraquinones. *Vitex negundo* and *Euphorbia hirta* tested negative for the presence of alkaloids and only *Vitex negundo* tested negative for the presence of cardiac glycosides (Table 1).

DISCUSSION

Phytochemical screening of the plants revealed some differences in the constituents of the four plants tested. *Acorus calamus* tested positive for all the phytochemicals tested; *Vitex negundo* showed the absence of anthraquinones, alkaloids and cardiac glycosides; *Coleus forskohlii* tested positive for all except anthraquinones while *Euphorbia hirta* tested positive for all except Anthraquinones and alkaloids. All the plants exhibited potent antioxidant activity (Figure 1). The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.[12] The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow.

The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The crude extract of *Euphorbia hirta* appeared to be as potent as Vitamin C with a maximum inhibition of 86.1% at 0.5mg/ml which is comparable to 95% for vitamin C at the same concentration. *Vitex negundo* was less potent as78.4% than the standard with a maximum inhibition of 95% at 1 mg/ml, followed by *Acorus calamus* which was also less potent (than vitamin C) with a maximum inhibition of 79.6% at 5mg/ml. *Coleus forskohlii* was the least potent showing a maximum inhibition of 71.7% at 5 mg/ml. This study suggests that these plants possess antioxidant activities which can counteract the oxidative damage induced by the malaria parasite. This may be one of their mode of action in malaria therapy.

Table 1: Phytochemical constituents of *Vitex negundo, Acorus calamus, Euphorbia hirta, Coleus forskohlii*

Test	*Acorus calamus*	*Vitex negundo*	*Coleus forskohlii*	*Euphorbia hirta*
Reducing Sugar	+	+	+	+
Anthraquinone	+	-	-	-
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tanins	+	+	+	+
Alkaloids	+	-	+	-
Cardiac Glycosides	+	-	+	+

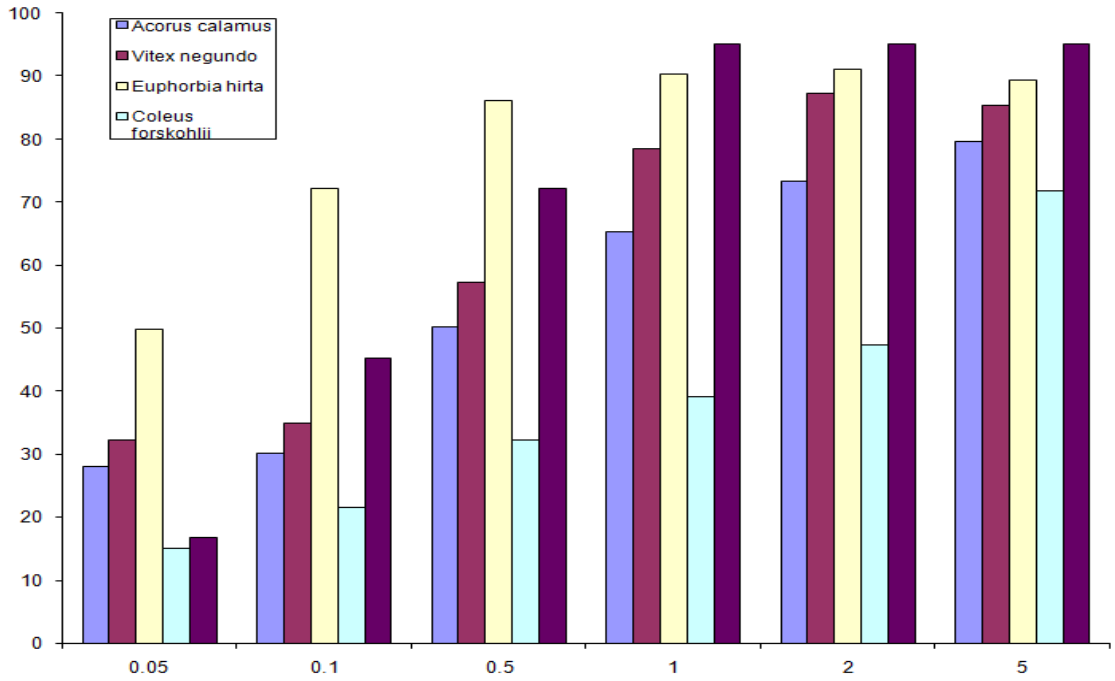

Figure 1: Inhibition of DPPH by the ethanolic extracts of *Vitex negundo, Acorus calamus, Euphorbia hirta, Coleus forskohlii*

CONCLUSION

Extracts from *Vitex negundo*, *Acorus calamus*, *Euphorbia hirta*, *Coleus forskohlii* showed varying antioxidant (free radical scavenging) activities when compared to vitamin C in the following order: *Coleus forskohlii* < *Acorus calamus* < *Vitex negundo* <Euphorbia hirta <Vitamin C. The results suggest that the antioxidant activity of these plants may contribute to their claimed antimalarial property.

Acknowledgement
The authors are thankful to Dr.Alok Lehri, Scientist E-1, Central Instrumental Facility at National Botanical Research Institute, Lucknow, for the authentification of plant material used in this study.

REFERENCES

[1] OPG Nmorsi; NCD Ukwandu; AO Egwunyenga. *Afr. J. Microbial Res*, **2007**, 61-64.
[2] R Tran; S Soc. *Trop. Med. Hyg*, **2000**, 94(1), 51-590.
[3] AG Kulkarni; AN Suryakar, AS Sardeshmukh, DB Rathi. *Ind. J. Clin. Biochem*, **2003**, 18(2), 136-149.
[4] PG Kremsner; B Greve; B Lell; D Luckner; D Schmidt. *Lancet*, **2000**, 355, 40-41.
[5] BS Das; NK Nanada. *Roy. Soc. Trop. Med. Hyg*, **1999**, 93, 58- 62.
[6] P Loria; S Miller; M Foley; L Tilley. *Biochem. J*, **1999**, 339, 363-370.
[7] TO Odugbemi; RA Odunayo; IE Aibinu; OP Fabeku. *Afr. J. Trad. CAM*, **2007**, 4(2), 191-198.
[8] A Sofowora. Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan. **1993**; pp 150.
[9] GE Trease; WC Evans. Pharmacognosy. 13th edn. Bailliere Tindall, London, **1989**, pp 176- 180.
[10] WB Williams; ME Cuvelier; C Berset. *Lebensmittel Wissenschaft und Technologie*, **1995**, 28, 25-30.
[11] GA Ayoola; T Sofidiya; O Odukoya; HAB Coker. *J. Pharm. Sci. & Pharm. Pract*, **2006**, 8, 133-136.
[12] O Polterait. *Current Org. Chem*, **1997**, 1, 415-440.