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Research Article

A Comparative Evaluation of *Swertia* Species as Potential Substituent of *Swertia chirata*

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ABSTRACT

The comparative chemical profile has been generated through TLC fingerprints of five *Swertia* species *viz*. *Swertia angustifolia* Buch Ham. ex D. Don, *S. chirata* Buch Ham, *S. cordata* (G. Don) C.B.Clarke, *S. lurida* (D. Don ex G. Don) C.B. Clarke and *S. purpurascens* (D. Don) A. Wall ex E.D. Clarke. TLC fingerprint of *Swertia* species showed a close chemical relationship between *S. chirata* and *S. lurida. In vitro* antimalarial activity against *Plasmodium berghei* test model in mice and antioxidant activity using DPPH assay have been performed. *S. lurida* and *S. chirata* showed maximum antimalarial activity at two different dose levels of 50 and 100 µg/ml using chloroquine (10 µM) as standard. However, only methanolic extract of *S. chirata* and *S. lurida* exhibited maximum *in vitro* antioxidant activity in DPPH assay shown IC_{50} 16.46 and 32.7 respectively using ascorbic acid as standard. The only species exhibiting better and comparable activity than *S. chirata* was found to be *S. lurida.* Thus *S. lurida* act as the best substitute of *S. chirata* in malarial fever.

Keywords: *Swertia*, Antimalarial and Antioxidant activity, *Plasmodium berghei.*

INTRODUCTION

Malaria is a life threatening and probably one of the oldest diseases known to mankind. *Plasmodium falciparum*, *P. malariae*, *P. vivax* and *P. ovale* are the common parasites causing malaria.[1] There were estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths mostly of children under 5 years and 109 countries were endemic for malaria in 2008, of which 45 were within the WHO African region. Current estimates place the clinical caseload between 300 and 500 million people annually and nine out of ten of these cases occur in sub-Saharan Africa. The most dangerous of all the human malarial parasites is *P. falciparum*. The reason for majority of infections reported in Africa is the climatic, other conditions.[2] The most commonly used drugs are quinoline based antimalarials which include quinine from *Cinchona succiruba* and its derivatives chloroquine, amodiaquine and mefloquine. Artemisinin from *Artemisia annua* and its derivatives artemether, arteether, artesunate and artelinic acid are the latest potential antimalarial agents. [3-4] Resistance to all known antimalarial drugs, with the exception of the artemisinin derivatives has developed to various degrees in several countries. [5] The need for the discovery of drugs which are effective against drug-resistant strains of malaria has been accelerated over the last two decades. Since two most potent and widely used antimalarial drugs quinine and artemisinin are from natural sources, there is always a great interest to look for new leads from plant sources. *Swertia chirata* (Family Gentianaceae) commonly known as 'chirata', a highly reputed plant of Ayurveda has been widely employed in herbal medicine for malarial fevers, [6-7] *Swertia* species are used in diseases of hepatobiliary system and act as a bitter tonic and febrifuge. [8-9] The different species of *Swertia* have been pharmacologically evaluated to scientifically validate various traditional claims of the plant *viz.* anti-inflammatory, [10] cardiovascular, [11] antimicrobial, [12] antioxidant, [13-15] antitumor, [15] hepatoprotective, [16-17] antidiabetic, [18] chemopreventive effects[19] and antimalarial activity.[20] The phytochemical investigations of the genus *Swertia* have yielded approximately 200 compounds with varying structural patterns till date. Xanthones, xanthone glycosides, iridoids/secoiridoids and triterpenoids constitute major classes of compounds reported from genus *Swertia*. [9] The most potential specie *Swertia* genus (*S.chirata*) is now nearly extinct from India. Hence, present study was planned to evaluate other commonly available species of *Swertia* for their antimalarial potential with a view to explore their value as a substitute of *S. chirata*.

MATERIAL AND METHODS

Identification of plant material*:* The whole plant part of *Swertia* species was collected from Sirkunda Devi (Mussoorie, Uttarakhand, India) situated at an altitude of 2050 meters. The identity of all plants *viz.* *S. angustifolia*, *S. chirata*, *S. cordata*, *S. lurida* and *S. purpurascens* was confirmed by studying the taxonomical

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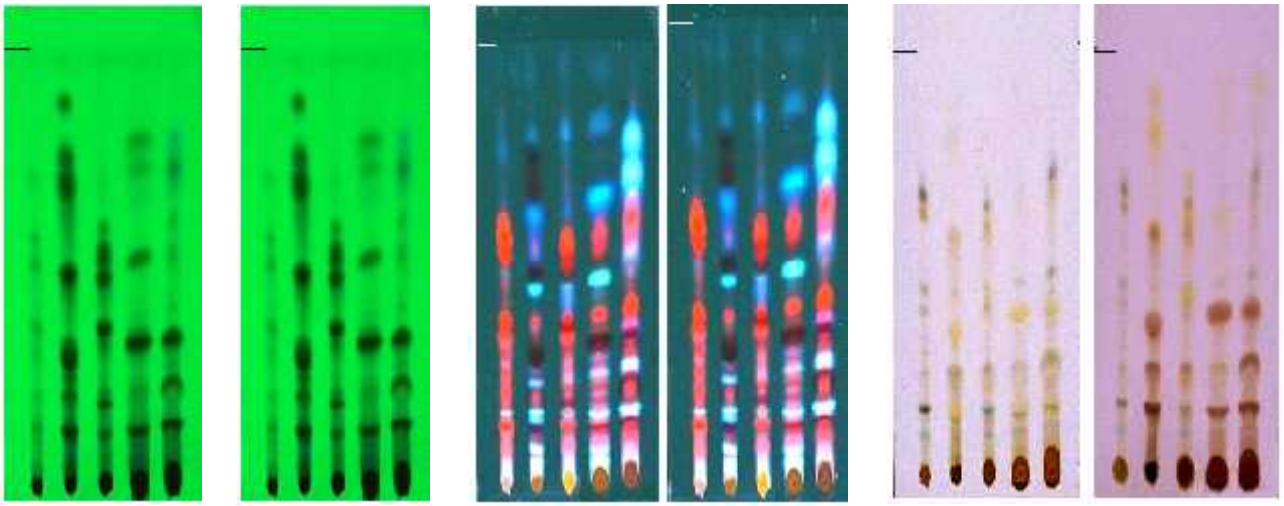

Fig 1: TLC fingerprint profile of methanolic extract of different Swertia species 1. S. angustifolia 2. S. chirata, 3. S. cordata, 4. S. lurida, 5. S. purpurascens, before and after spraying with 10 % aqueous KOH solution and visualization under 254 nm, 366 nm and white light. Solvent system: Toluene : Acetone (9.5: 0.5)

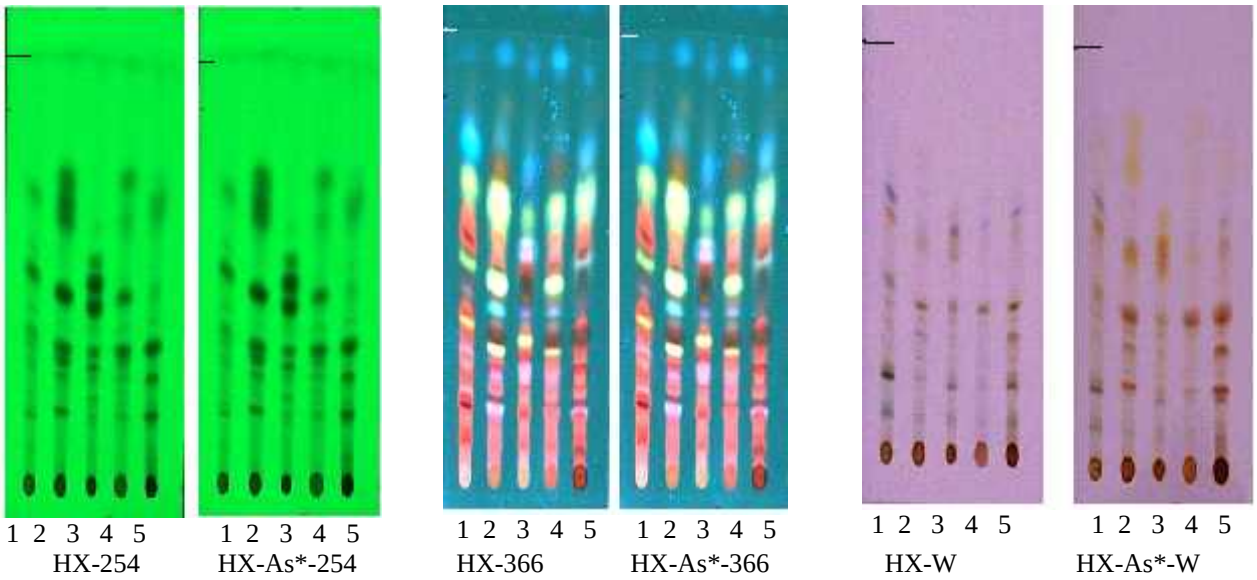

Fig 2: TLC fingerprint profile of hexane extract of different Swertia species 1. S. angustifolia, 2. S. chirata, 3. S. cordata, 4. S. lurida 5. S. purpurascens, before and after spraying with 10 % aqueous KOH solution and visualization under 254 nm, 366 nm and white light. Solvent system: Toluene : Acetone (9.5: 0.5

characters and comparing with herbarium specimens available in the museum-cum-herbarium of the University Institute of Pharmaceutics Sciences, Panjab University, Chandigarh. A voucher specimen of each plant was deposited in the museum vide reference numbers 1462, 1458, 1459, 1463 and 1144 respectively.

Extraction: The plants after collection were dried in shade. Hexane, chloroform and methanolic extracts of all the five *Swertia* species were prepared for evaluation of antimalarial and antioxidant activity evaluation. A 10 g powdered material of each plant was defatted with petroleum ether and marc was refluxed with hexane on water bath at 40-50^{0}C for 6 h to obtained hexane fraction. In successively partition manner was used to obtained chloroform and methanolic extracts under similar conditions. The percentage yield of total hexane, chloroform and methanolic extracts was 1.4, 1.4 and 10 % w/w respectively. All extracts were dried under reduced pressure and stored in vacuum desiccator till further use.

Animals*:* Adult Swiss mice, BALB/ c strain, 25-30 g of either sex, bred in the Central Animal House of Panjab University, Chandigarh were used. Animals were fed on the standard diet and water *ad libitum*. All animal procedures described were reviewed and approved by the University Animal Ethical Committee.

Preparation of doses: For antimalarial activity, extracts were dissolved in minimum amount of dimethyl sulfoxide (20 mg/ml) and diluted with RPMI to get 50 and 100 µg/ml doses. The suspended samples were then incubated with infected blood (1 % parasitaemia) for 21 h. [21] Normal control which contained infected blood in complete medium was also subjected to similar conditions along with the test extracts.

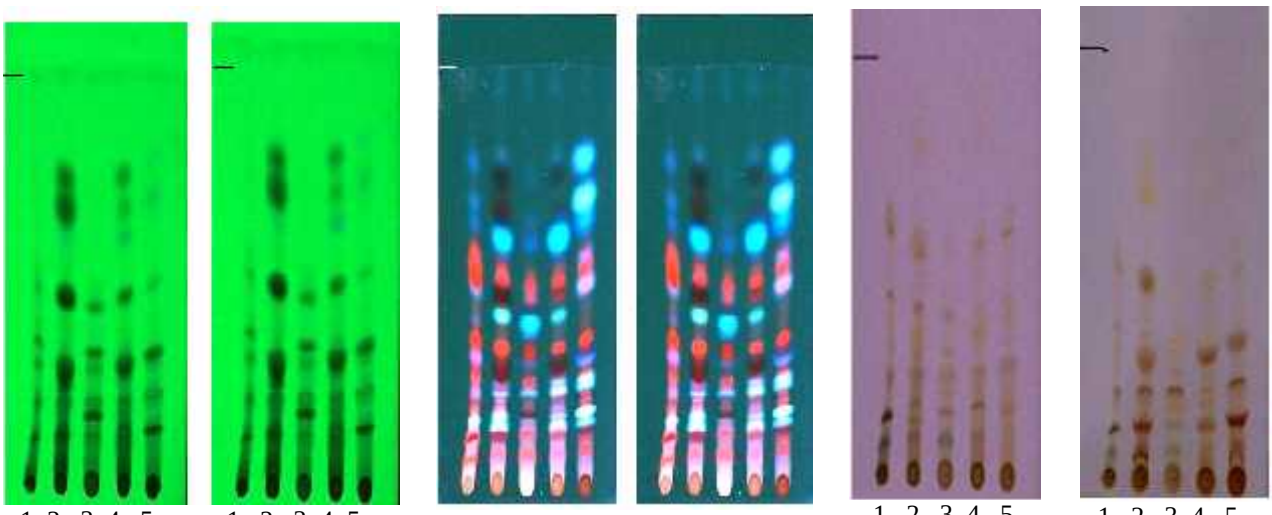

Fig 3: TLC fingerprint profile of chloroform extract of different Swertia species 1. S. angustifolia, 2. S. chirata, 3. S. cordata, 4. S. lurida, S. purpurascens, before and after spraying with 10 % aqueous KOH solution and visualization under 254 nm, 366 nm and white light. Solvent system: Toluene : Acetone (9.5: 0.5)

Table 1: Free radical scavenging effect of ascorbic acid in DPPH assay

Concentration (µg/ml)	Absorbance	% inhibition
2	0.060	29.41
10	0.052	39.22
25	0.040	52.66
50	0.021	76.92
75	0.007	91.76

Ascorbic acid, y= 0.86x + 30.13, R^2 = 0.9, Abs of control = 0.075

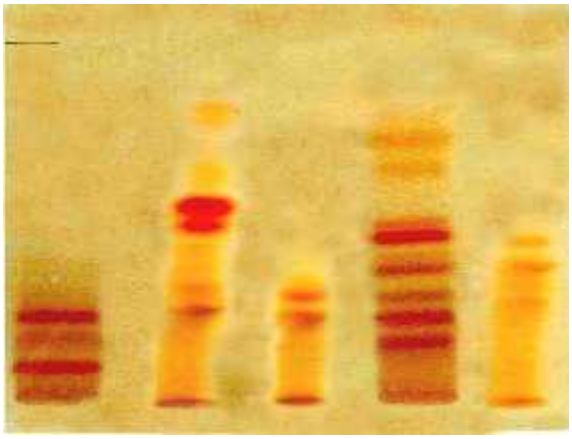

Fig 4: TLC fingerprint profile of methanolic extract of different Swertia species 1. S. angustifolia, 2. S. chirata, 3. S. cordata, 4. S. lurida, S. purpurascens, showing presence of bitter constituents after spraying with Fast-red-salt reagent solution and visualization under white light. Solvent system: Chloroform : methanol (8 : 2)

TLC fingerprint profile**:** About 5 g of powdered material of all the five *Swertia* species *viz. S. angustifolia, S. chirata, S. cordata, S. lurida* and *S. purpurascens* was extracted using soxhlet apparatus for 5 h separately with 100 ml each of methanol, hexane and chloroform to obtain methanol soluble, hexane soluble and chloroform soluble portions. Each extract was concentrated under reduced pressure in rotary vacuum evaporator (Eyela, NE-10, Japan) and reconstituted with appropriate solvents(s) to develop TLC fingerprint profile of hexane and chloroform methanolic extracts. The pre-coated silica gel G plates (E. Merck, alumina base) were activated at 110 ^{0}C for 30 min in an oven before use and after development in suitable solvents; the number and position of spots were visualized by spraying the plate with 10 % aqueous KOH/anisaldehyde-sulphuric acid reagent, Fast-red-reagent or observing the plate under ultraviolet light. All solvents used were of GR grade (E. Merck) and large number of solvent systems were tried and used for TLC studies. The various solvent systems, toluene : acetone (9.5:0.5) was finally selected for preparing TLC fingerprint profile as it showed maximum resolution.

In-vitro antimalarial activity: *In vitro* antimalarial evaluation was done according to WHO, 2001 guidelines.[22] A suspension (0.9 ml) of *P. berghei* infected red blood cells (0.1 % parasitaemia, 3-5 % cell hematocrit) was added to wells of standard 96 well tissue culture plate (Laxbro) containing different doses of test extracts and control to be tested. Chloroquine (10 µM) was used as positive control. Microtiter plates were incubated for 21 h at 37 ^{0}C in a sealed candle jar. The assay was terminated by aspirating the medium and smears were prepared, air dried and fixed in methanol (2 min). The smeared slides were stained with Giemsa solution (30 min), washed under running water, air dried

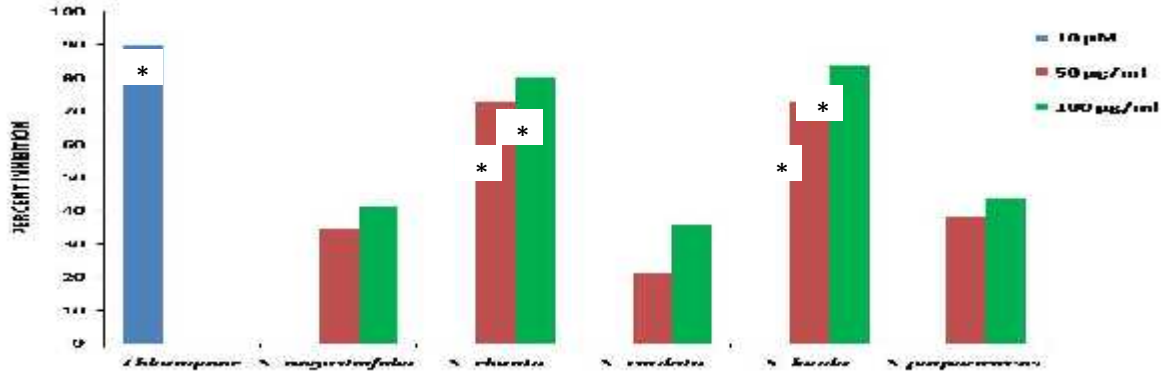

Fig 5: *In vitro* antimalarial evaluation of methanolic extract of different species of *Swertia*.

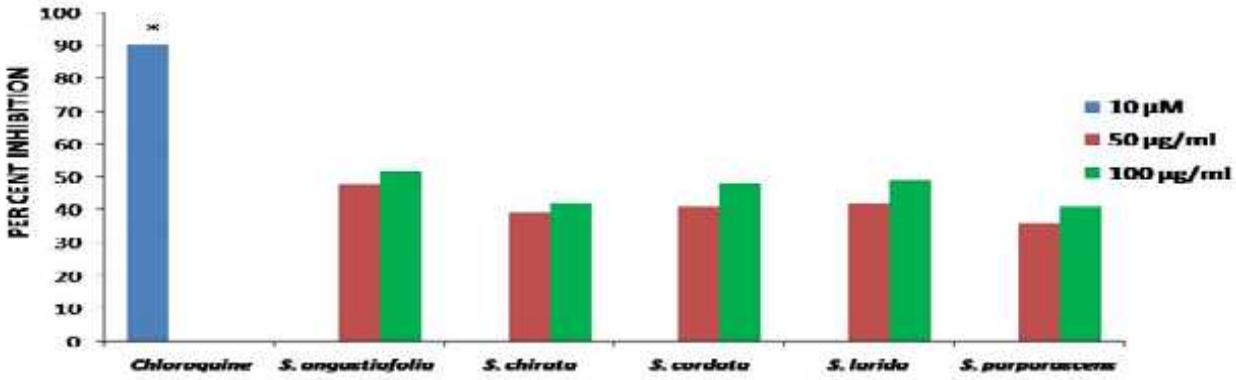

Fig 6 : *In vitro* antimalarial evaluation of hexane extract of different species of *Swertia*.

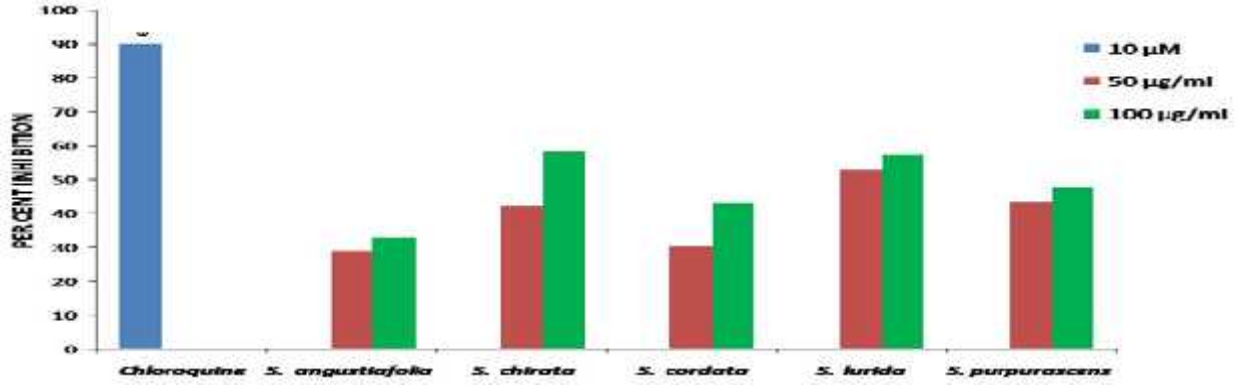

Fig 7 : *In vitro* antimalarial evaluation of chloroform extract of different species of *Swertia.*

and observed under microscope. The number of parasites per 500 RBC's was counted. All the samples were tested at two different dose levels 50 and 100 µg/ml and results were expressed as percentage invasion inhibition using the following formula: Per cent invasion inhibition =

$$100 - \frac{\text{No of rings in standard/test}}{\text{No of rings in control (21 h)}} \times 100$$

In-vitro antioxidant activity: An antioxidant activity of the extracts of all five *Swertia* species *viz S. angustifolia*, *S. chirata*, *S. cordata*, *S. lurida* and *S. purpurascens* was determined *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Ascorbic acid was used as a standard and stock solution (200 µg/ml) for all the extracts was prepared in ethanol. A fixed volume of 1 ml of various concentrations of all the extracts was added to 1 ml of DPPH (100 µM) and volume was made up to 4 ml with ethanol. All reaction mixtures were kept in dark for 25-30 min period at room temperature and absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in per cent was calculated according to the formula. [23]

Per cent inhibition =

$$\frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100$$

Where A_{Blank} is the absorbance of the control reaction (containing all reagents except the test extracts) and A_{Sample} is the absorbance of the test extracts. The graph was plotted between concentration of the test extracts *vs* per cent inhibition of free DPPH radicals by the test extracts and 50 % inhibition (IC_{50}) was calculated as a measure of antioxidant activity.

RESULTS

TLC profile: A comparative TLC fringerprint profile of methanolic, hexane and chloroform extracts for all the five *Swertia* species had been generated by using best resolved solvent system and visualized under 254 nm, 366nm and under normal white light after spraying with

10 % KOH solution and Fast-red reagent are generated. The figure (1,2,3) show presence of different xanthones under 254 nm under white light and, secoiridoids and triterpenoid under 366 nm. The figure 4 shows presence of bitter principles of *Swertia* species when spray with Fast-red-salt reagent and visualization under white light. TLC fringerprint profile show presence of maximum number of spots in *S.chirata* and *S. lurida* along with chemical similarities.

Antimalarial activity: Antimalarial activity was observed for whole plant of five different species of *Swertia*. Three different extarcts *viz.* methanolic, hexane and chloroform were tested at two different dose levels of 50 and 100 µg/ml. The results showed (Figure 5) that 100 µg/ml dose of methanolic extract of *S. lurida* and *S. chirata* exhibited maximum antimalarial activity against *P. berghei* with percentage inhibition of 83.7 and 79 respectively. A close look at figure 5-7 revealed that only methanolic, extracts of *S. lurida* at 50 and 100 µg/ml dose levels exhibited maximum percentage invasion inhibition of 72.7 and 72.7 respectively against 90 % inhibition of schizont maturation shown by chloroquine (10 µM).

Antioxidant activity: *In vitro* antioxidant activity of different extract of all five *Swertia* species was performed using DPPH assay and the results are shown in (Table 2). The concentration of each extract providing 50 % inhibition (IC_{50}) was calculated from graph plotted between percentage inhibition against concentration of the extract used and standard (figure 8 and 9). The results showed (Table 1) that methanolic extract of *S. chirata* and *S. lurida* exhibited maximum antioxidant activity at IC_{50} 16.46 and 32.7 respectively, using ascorbic acid as standard (Table 2). Hexane and chloroform extracts of all the *Swertia* species does not exhibit antioxidant activity.

DISCUSSION

Xanthones and secoiridoids are main active principles of

Table 2: IC_{50} values of various extracts of different species of *Swertia* in DPPH assay.

Sr No	*Swertia* species	Extract	IC_{50}
1	*angustifolia*	Methanolic	119.9
2	*S. chirata*	Methanolic	16.46
3	*S. cordata*	Methanolic	87.3
4	*S. lurida*	Methanolic	32.7
5	*S. purpurascens*	Methanolic	77.6

Standard, IC_{50} = 23.1

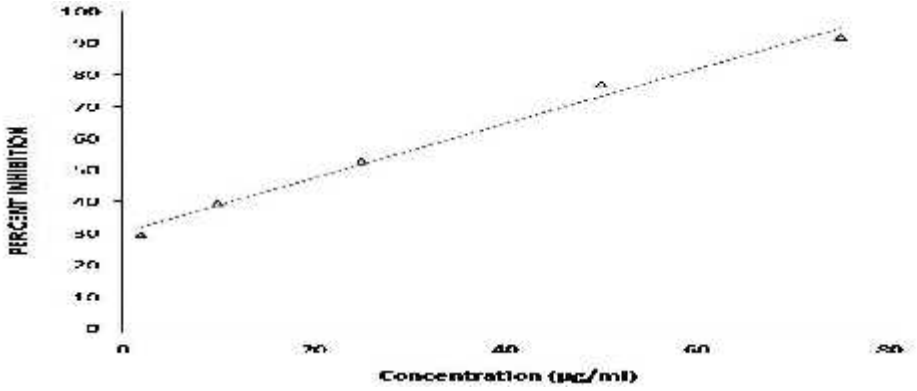

Fig 8: Free radical scavenging effect of ascorbic acid (standard) in DPPH assay.

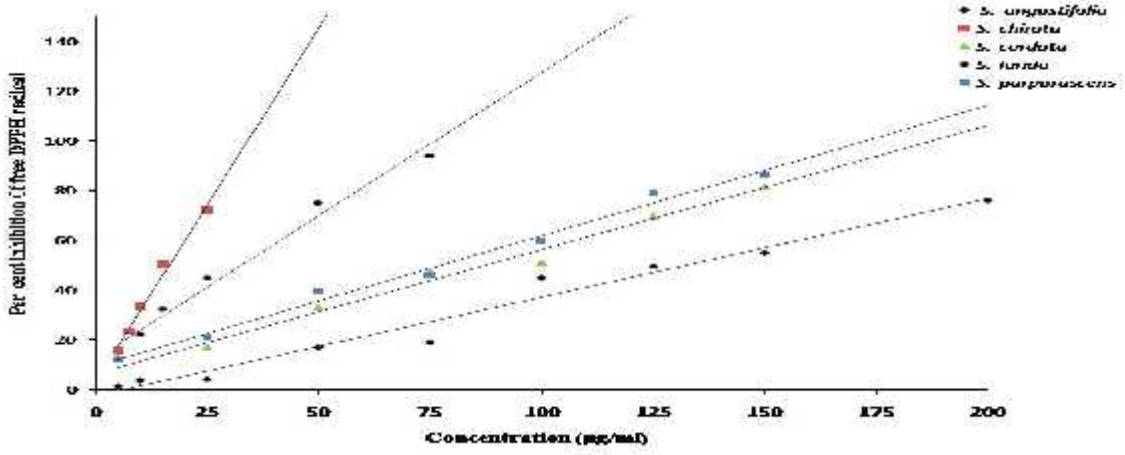

Fig 9 : Free radical scavenging effect of total methanolic extract of different species of Swertia in DPPH assay.

Swertia species.[9] The comparative TLC fingerprint profile of different extracts of all the five species of *Swertia* was generated and an attempt has been made to elaborate the similarities and dissimilarities in chemical profile between various species. The chemical profile shows presence of xanthones, bitter principles in *Swertia* species. *S. chirata* and *S. lurida* were found to have many similarities and more number of spots in their chemical profile on the basis of TLC fingerprinting. The antioxidant evaluation of all *Swertia* species was done using DPPH assay. The methanolic extract of *S. chirata* and *S. lurida* showed promising activity. *Swertia* species attributed to antimalarial activity. [20] Xanthones and antioxidant compounds work together in synergizing manner and increase a fold in antimalarial activity. [24-26] The antimalarial potency of the hydroxyxanthones correlated well with their ability to inhibit *in vitro* heme polymerization, suggesting that xanthones exert their antimalarial action by preventing hemozoin formation.[27-28] An antimalarial evaluation of all *Swertia* species was also performed. Two species, *S. lurida* and *S. chirata* showed maximum inhibition in antimalarial activity against *P. berghi*. Thus presence of active principles and antioxidant in both the species strength there traditional claim as antimalarial drug.[29]

CONCLUSION

The most potential species *S. chirata* is now nearly extinct from India. The present investigations clearly demonstrate that *S. cordata*, *S. angustifolia* and *S. purpurascens* cannot be used as substituents for *S. chirata*. The only species exhibiting better and comparable activity than *S. chirata* was found to be *S. lurida* which also have similarities in chemical profile. Thus propose *S. lurida* as the best substitute of *S. chirata* in malarial fever.

ABBREVIATIONS

CH: Chloroform extract without spray, CH-As*: Chloroform extract after spray, CH-As*-W: Chloroform extract after spray under white light, HT: Hexane extract without spray

HT-As*: Hexane extract after spray, HT-As*-W: Hexane extract after spray under white light, MT: Methanolic extract without spray, MT-As*: Methanolic extract after spray, MT-As*-W: Methanolic extract after spray under white light

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