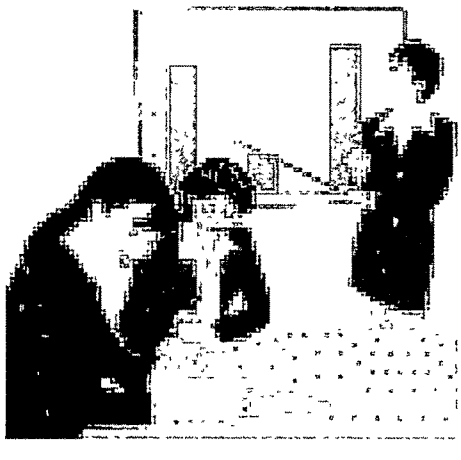


RESULTS

3.1 PHARMACOGNOSTIC STUDIES

3.1.1 Macroscopical examination

Flower heads of ***Sphaeranthus indicus,*** were violet in color when fresh, after drying under shade they turned dark brownish-violet (Fig 3.1). Diameter of flower heads was in the range of 1- 1.5 cm. Flower heads were soft to touch and became slightly rough on drying. Odor was pleasant and aromatic. Taste was bitter.

Roots of ***Cissampelos pareira*** were wrinkled, yellowish brown in color (Fig 3.2). Taste was slight bitter and characteristic. Roots were broken with fibrous fracture. Length of roots was ranging from 10- 15 cm.

Rhizomes of ***Curculigo orchioides*** were small pieces of length ranging from 1.5- 2.5 cm. Color of rhizomes was dark brown outside and off white inside (Fig 3.3). Rhizomes were broken with fine and short fracture. Taste was mucilaginous.

3.1.2. Microscopic examination

3.1.2.1 Transverse section (T.S.) of intact drugs

T.S. of root (Fig 3.4) of ***C. pareira*** revealed presence of six to eight layers of brown colored cork. Cork several layered with tabular cells (Width 30-42 microns and length 40- 65 microns) outer layer was filled with brown contents and inner layer was colorless. Phelloderm three to five layered below cork, made up of parenchymatous cells whose corners were thickened. Some cells contain minute starch grains (10- 15 microns). Cambium was followed to cortex, reacted to phloroglucinol hydrochloric acid to give pink color. Medullary rays distinct bi to multiserial, parenchymatous, in continuation with those of xylem. Medullary rays (Width 30- 40 microns and length 90- 100 microns) were slightly narrower in xylem region while wider in phloem region. Xylem was well represented, divided by large medullary rays at regular intervals consists, vessels, fibres. Phloem tissue

was reacted with phloroglucinol and hydrochloric acid to give pink color. Metaxylem was observed. Medullary rays were three to five cells wider.

T.S. of rhizome (Fig 3.5) of ***C. orchioides*** revealed the typical character of rhizome. Dark brown colored cork cells (Width 25- 35 microns and length 35- 40 microns) were arranged tightly. Cortex was composed of loosely arranged rounded cells with intercellular spaces and abundant starch. Some of the cells of cortex contained ca-oxalate crystals. Starch grains also observed when T.S. was treated with iodine and sulfuric acid. Interspersed with cortical parenchymatous cells were found numerous vessels and vascular bundles. In the lower part of T.S. pericyclic fibres were observed they reacted with phloroglucinol and hydrochloric acid to give pink color. Medullary rays were not clearly visible.

3.1.2.2 Microscopical features of powdered crude drugs

Powder of flower heads of *S. indicus* principally showed presence of large arrow shaped structures identified as fibres. These fibres showed pink contents when treated with phloroglucinol and hydrochloric acid to give pink color. Numerous clusters of epidermal cells also observed. Cells containing ca-oxalate crystals were also found. Long unicellular covering trichomes were also noted. Compound starch grains were present. Numbers of vessels were present (Fig 3.6).

Powder of roots of *C. pareira* when observed under microscope showed clusters of cork cells with dark brown contents. Pieces showing cells of medullary rays were present. Vessels and fibres were present. Pitted vessels also noted. Mass of cells from cortex having thickened walls were also found. Starch grains were simple (Fig 3.7).

Powder of rhizomes of *C. orchioides* showed presence of compound starch grains, cork cells, mass of cells from cortex, calcium oxalate crystals (needle shaped), fibres and ground tissues (Fig 3.8).

Fig 3.1: Photograph of flower heads of *Sphaeranthus indicus* Linn.

Fig 3.2: Photograph of roots of *Cissampelos pareira* Linn.

Fig 3.3: Photograph of rhizomes of *Curculigo orchioides* Gaertn.

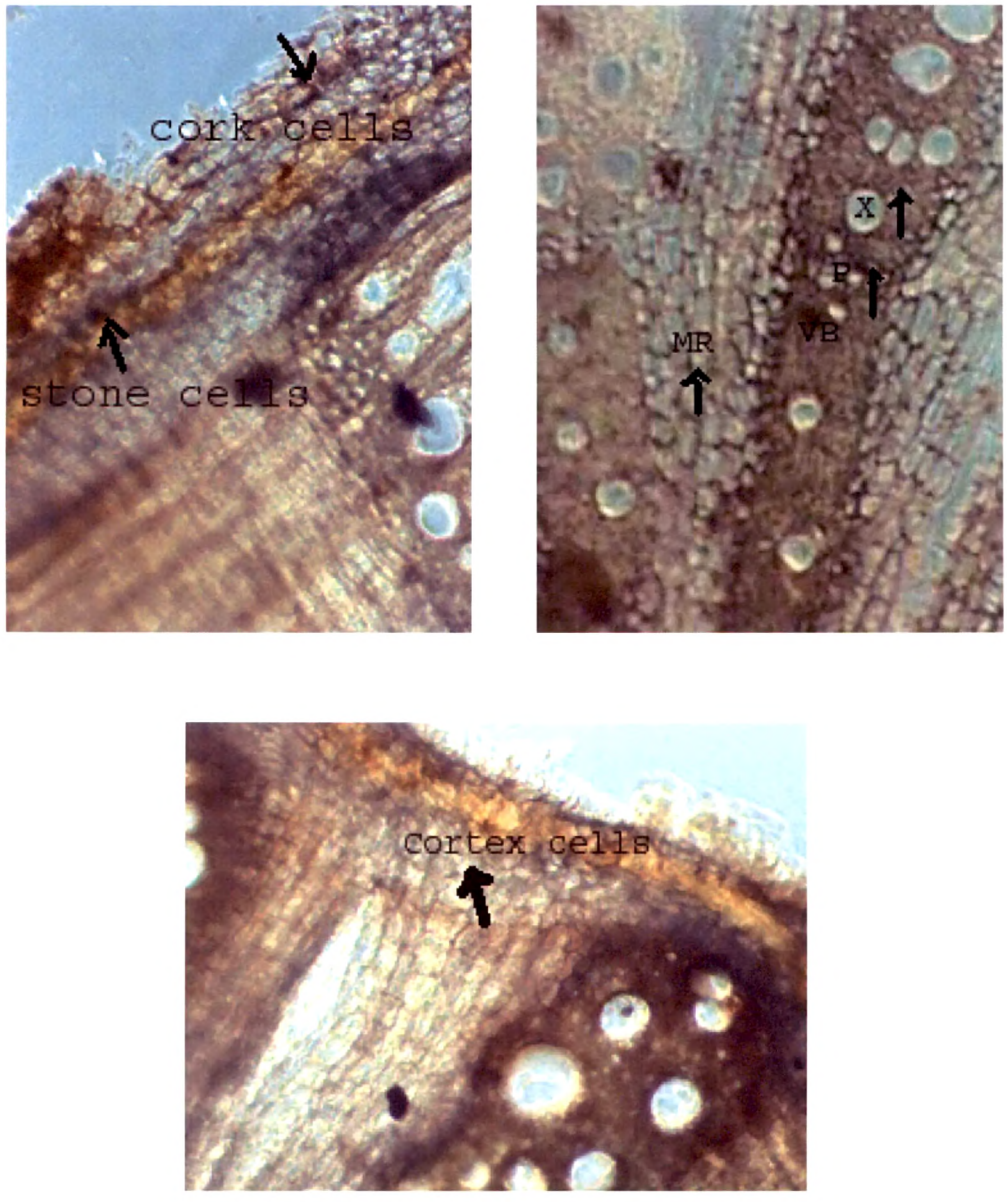


Fig 3.4: Transverse section of root of *C. pareira* (Magnification 10X)

X- Xylem, MR- Medullary rays, P- Phloem, VB- Vascular bundles

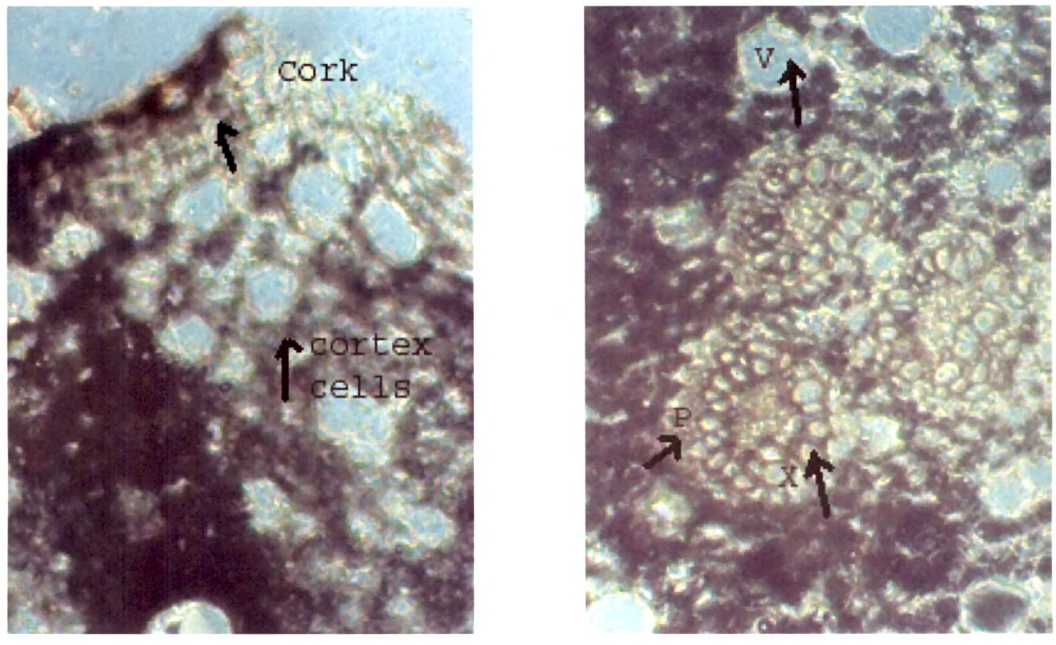


Fig 3.5: Transverse section of rhizome of *C. orchioides.*

(Magnification 10X)

V- Vessels, P- Phloem, X- Xylem

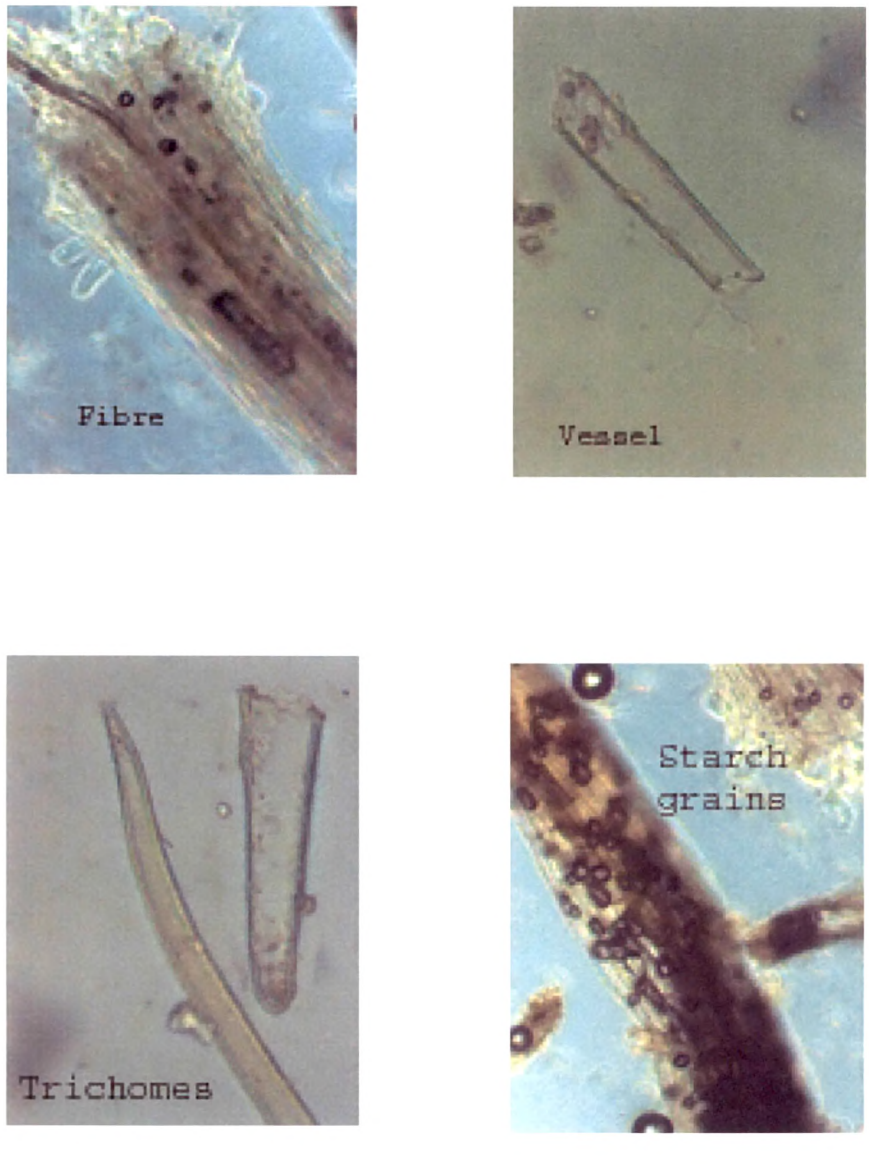


Fig 3.6: Microscopical characteristics of powdered flower heads of *S.indicus* (Magnification 40X)

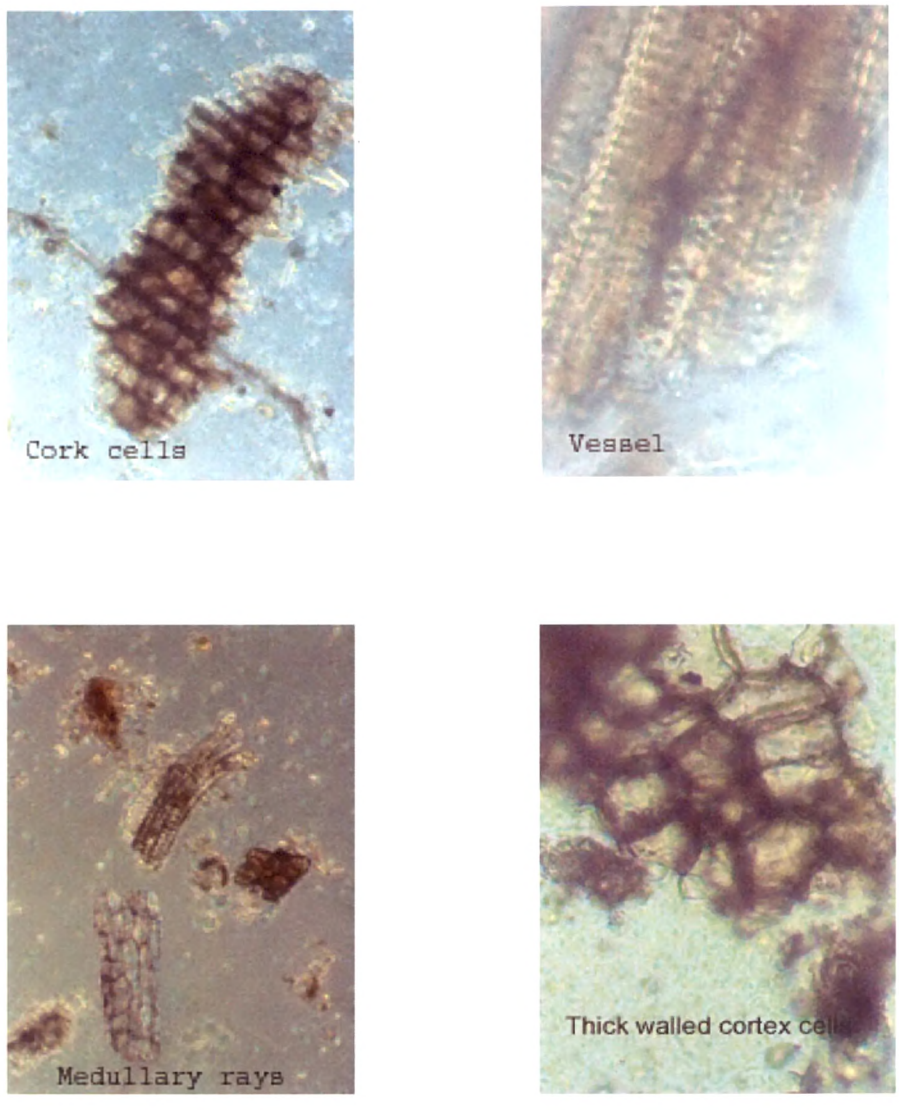


Fig 3.7: Microscopical characteristics of powdered roots of *C. pareira* (Magnification 40X)

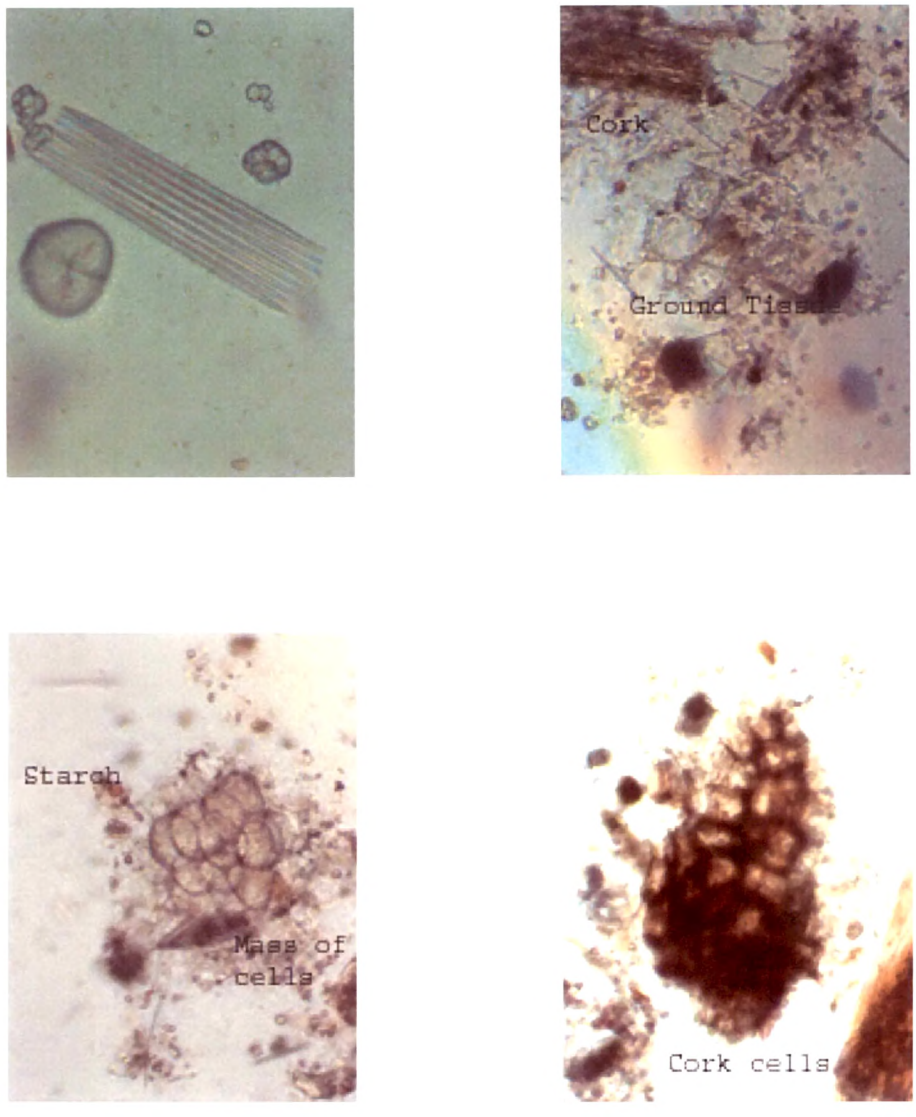


Fig 3.8: Microscopical characteristics of powdered rhizomes of *C. orchioides* (Magnification 40X)

3.1.3 Proximate analysis

The results obtained from various determinations are compiled in Table 3.1 being the average of three readings taken. Foreign organic matter was found more for the plant *C. pareira,* followed by *S. indicus* and *C. orchioides.* Highest moisture content was found in flower heads of *S. indicus* i.e. 9.38 %, while other plants (*C. pareira*- 2.85 % and *C. orchioides*- 2.50 %) showed negligible moisture content. Water soluble extractives found more in percentages than alcohol soluble extractives for all the three plants selected. It indicates presence of more water soluble components in plants.

Total ash was more in *C. orchioides* (15.26 %) followed by *C. pareira* (13.59 %) and *S. indicus* (10.25 %). Acid insoluble ash, which is indication of contamination of drug with silicacius matter, was more in *C. pareira* (5.89 %), followed by *S. indicus* (3.96 %) and *C. orchioides* (3.58 %). Water soluble ash followed the order *S. indicus* (5.65 %), *C. pareira* (6.32 %) and *C. orchioides* (8.65 %).

3.2 PHYTOCHEMICAL STUDIES:

3.2.1 Preliminary phytoprofiles:

All the selected plants were subjected to process. The different extracts obtained with their % yield, color and consistency are recorded in Table 3.2. Results indicate maximum extractive values attained with polar solvents like methanol and water in all the selected plants, whereas with non polar solvents like petroleum ether, benzene, chloroform and ethyl acetate these extractive values were very less.

3.2.2 Qualitative chemical tests:

The extracts obtained in the successive extraction process were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents in prepared extracts.

S. indicus showed presence of alkaloids, steroids, terpenoids, flavonoids, fats, phenolics and tannins. *C. pareira* showed presence of

alkaloids, steroids, terpenoids, flavonoids, fats, phenolics and tannins. *C. orchioides* showed presence of steroids, terpenoids and phenolics. All the three plants showed presence of carbohydrates and amino acids and absence of anthraquinones. Results are summarized in Table 3.3.

3.2.3 TLC profiles of the extracts obtained by successive solvent extraction

The extracts obtained in the successive extraction process were subjected to determine the presence of various phytoconstituents by spraying different detecting agents. Rf of the compounds reacted to these reagents were then recorded (Table 3.4, 3.5, 3.6).

3.2.4 Fractionation of extracts

Results of fractionation of methanol extracts of *S. indicus, C. pareira* and *C. orchioides* summarized in the Table 3.7.

Methanol extract of *S. indicus* was found to contain as much as 39 % of non polar components extracted by solvents like petroleum ether, benzene and chloroform, whereas major share was of polar compounds (58%) extracted by methanol.

Methanol extract of *C. pareira* contained 15.56 % alkaloids. Non alkaloidal compounds shared major percentage (84.44 %).

Methanol extract of *C. orchioides* showed negligible content of non polar compounds (8.18 %), while polar components had share of 88.50 %. Due to this lower yield of non polar compounds, total methanol extract was used further for screening of activities.

Table 3.1: Proximate analysis of selected plant drugs.

Determinations (% w/w)	Plant drugs		
	S. indicus **(Flower heads)**	***C. pareira*** **(Roots)**	***C. orchioides*** **(Rhizomes)**
Foreign organic matter	10.00%	15.00%	9.50 %
Moisture content	9.38 %	2.85 %	2.50 %
Total ash	10.25 %	13.59 %	15.26 %
Acid insoluble ash	3.96 %	5.89 %	3.58 %
Water soluble ash	5.65 %	6.32 %	8.65 %
Alcohol soluble extractives	7.00 %	8.5 %	6.00 %
Water soluble extractives	12.00 %	15.00 %	8.00 %

n= 3.

Table 3.2: Preliminary phytochemical screening of selected plant drugs.

Sr.No.	Solvent used	Plant drugs					
		S. indicus		***C. pareira***		***C. orchioides***	
		Color & Consistency	Average Extractive value % (w/w)	Color & Consistency	Average Extractive value % (w/w)	Color & Consistency	Average Extractive value % (w/w)
1	Petroleum ether (60-80^0)	Greenish-yellow, Semisolid, oily.	2.0	Greenish-yellow, Semisolid, oily.	2.25	Light brown, soft mass.	1.50
2	Benzene	Dark green, semisolid, sticky mass.	1.42	Dark greenish-yellow, Semisolid.	1.25	Brown, sticky mass.	1.65
3	Chloroform	Dark green, soft mass.	1.48	Yellow brown, semisolid mass.	1.00	Brown, sticky mass.	2.00
4	Ethyl Acetate	Yellow brown mass.	0.90	Yellow brown, semisolid mass.	0.85	Brown, solid powder.	3.50
5	Methanol	Coffee brown, semisolid mass.	6.5	Coffee brown, semisolid mass.	10.50	Dark brown, semisolid mass.	6.00
6	Water	Coffee brown, semisolid mass.	15.00	Dark coffee brown, semisolid mass.	12.37	Dark brown, semisolid mass.	7.5

Table 3.3: Qualitative chemical tests of different extracts of selected plants drugs.

Class of compounds	Plant drugs																	
	S. indocus						***C. pareira***						***C. orchiodes***					
	P	**B**	**C**	**E**	**M**	**W**	**P**	**B**	**C**	**E**	**M**	**W**	**P**	**B**	**C**	**E**	**M**	**W**
Alkaloids	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-	+	-
Carbohydrates	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+
Steroids/Terpenoids	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	-
Proteins & Amino acids	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+
Saponins	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+
Fixed oils/Fats	+	+	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-
Flavonoids	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-
Phenolics	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+

P- Petroleum ether extract
B- Benzene extract
C- Chloroform extract
E- Ethyl acetate extract
M- Methanol extract
W- Water extract
+- Present
- - Absence

Table 3.4: TLC profile of the extracts obtained by successive solvent extraction of *S. indicus*

Class of compound	Detection reagent	Solvent system	Extracts					
			P	B	C	E	M	W
Anthraquinones	5 % Alc. KOH	A	-	-	-	-	-	-
Carbohydrates	5 % H_2SO_4	B	-	-	-	-	0.26, 0.29 (Brown)	0.22, 0.30, 0.35 (Brown)
Alkaloids	Dragendorff's reagent	A	-	-	-	-	0.95 (Orange)	-
Flavonoids	UV-365 & NP-PEG reagent (Fluorescence)	A	-	-	0.32 (Yellow), 0.25 (Yellow), 0.19 (Orange), 0.11 (Orange-red).	0.50 (Yellow), 0.45 (Greenish), 0.35 (Orange), 0.20 (Yellow), 0.10 (Blue)	0.60 (Green), 0.52 (Yellow), 0.41 (Orange), 0.33 (Yellow), 0.23 (Yellow), 0.09 (Orange)	0.12 (Yellow), 0.05 (Orange)
Saponins/ Triterpenoids	VS reagent/ AS reagent	A	0.85, 0.78, 0.75, 0.65, 0.55, 0.45 (All spots dark blue colored)	0.90, 0.88, 0.85, 0.75, 0.68, 0.60, 0.50, 0.30 (Dark blue colored spots)	0.75, 0.65, 0.60, 0.55, 0.35, 0.25 (Dark blue colored spots)	0.52, 0.46, 0.40, 0.35, 0.22 (Dark blue spots)	0.45, 0.42, 0.38, 0.36, 0.25 (Dark blue spots)	0.30, 0.22, 0.10 (Dark blue/ Black spots)
Proteins and Amino acids.	Ninhydrin Reagent	B	-	-	-	-	0.50, 0.45, 0.30, 0.21, 0.01 (Pink colored spots)	0.45, 0.29, 0.20, 0.01 (Dark pink colored spots)

A: Ethyl Acetate: Methanol: Water (10:1.35:1.00); B: n-Butanol: Glacial acetic acid: Water (6:2:2)

P- Petroleum ether extract
B- Benzene extract
C- Chloroform extract
E- Ethyl acetate extract
M- Methanol extract
W- Water extract
+- Present
- - Absence

Table 3.5: TLC profile of the extracts obtained by successive solvent extraction of *C. pareira*

Class of compound	Detection reagent	Solvent system	Extracts					
			P	B	C	E	M	W
Anthraquinones	5 % Alc. KOH	A	-	-	-	-	-	-
Carbohydrates	5 % H_2SO_4	B	-	-	-	-	0.60, 0.42, 0.20 (Dark brown colored spots)	0.55, 0.46, 0.34, 0.22, 0.01 (Dark brown colored spots)
Alkaloids	Dragendorff's reagent	A	0.75, 0.56, 0.22 (Orange spots)	0.76, 0.56, 0.22 (Orange spots)	0.80, 0.75, 0.55, 0.24 (Orange spots)	0.80, 0.75, 0.55, 0.24 (Orange spots)	0.89, 0.75, 0.65, 0.58, 0.22, 0.09 (Orange spots)	0.11, 0.09 (Orange spots)
		B	0.98, 0.50, 0.20 (Orange spots)	0.53, 0.25 (Orange spots)	0.54, 0.28 (Orange spots)	0.22, 0.11, 0.09 (Orange spots)	0.76, 0.58, 0.35, 0.22, 0.11 (Orange spots)	0.50, 0.35, 0.11, 0.09 (Orange spots)
Flavonoids	UV-365 & NP-PEG reagent	A	-	-	-	-	-	-
Saponins/ Triterpenoids	VS reagent / AS reagent	A	0.89, 0.81, 0.75, 0.68, 0.62, 0.50, 0.45, 0.30 (Dark blue, violet, pink spots)	0.85, 0.81, 0.75, 0.60, 0.55, 0.45, 0.32, 0.30, 0.24 (Pink, dark violet spots)	0.98, 0.87, 0.79, 0.75, 0.65, 0.60, 0.54, 0.41, 0.32, 0.21 (Pink, reddish, violet spots)	0.75, 0.72, 0.68, 0.60, 0.50, 0.48, 0.33, 0.02 (Pink. Greenish, violet spots)	0.50, 0.35, 0.32, 0.30, 0.25, 0.01 (Pink, greenish spots)	0.35, 0.30, 0.25 (Dark violet spots)
Proteins and amino acids	Ninhydrin reagent	B	-	-	-	-	0.75, 0.61, 0.50, 0.32, 0.01 (Dark pink, violet spots)	0.70, 0.52, 0.35, 0.30, 0.25, 0.10 (Pink-reddish spots)

A: Ethyl Acetate: Methanol: Water (10:1.35:1.00), B: Toluene: Ethyl Acetate (7:3).

P- Petroleum ether extract
B- Benzene extract
C- Chloroform extract
E- Ethyl acetate extract
M- Methanol extract
W- Water extract
+- Present
- - Absence

Table 3.6: TLC profile of the extracts obtained by successive solvent extraction of *C. orchioides*.

Class of compound	Detection reagent	Solvent system	Extracts					
			P	B	C	E	M	W
Anthraquinones	5 % Alc. KOH	A	-	-	-	-	-	-
Carbohydrates	5 % H_2SO_4	B	-	-	-	-	0.50, 0.32, 0.22 (Dark brown colored spots)	0.45, 0.35, 0.21, 0.12, 0.01 (Dark brown colored spots)
Alkaloids	Dragendorff's reagent	A	-	-	-	-	0.85, 0.54 (Orange spots)	-
Flavonoids	UV-365 & NP-PEG reagent	A	-	-	-	-	-	-
Saponins/ Triterpenoids	VS reagent / AS reagent	A	0.89, 0.81, 0.75, 0.68, 0.62, 0.50, 0.45, 0.30 (Dark blue, violet, pink spots)	0.85, 0.81, 0.75, 0.60, 0.55, 0.45, 0.32, 0.30, 0.24 (Pink, dark violet spots)	0.98, 0.87, 0.79, 0.75, 0.65, 0.60, 0.54, 0.41, 0.32, 0.21 (Pink, reddish, violet spots)	0.75, 0.72, 0.68, 0.60, 0.50, 0.48, 0.33, 0.02 (Pink. Greenish, violet spots)	0.50, 0.35, 0.32, 0.30, 0.25, 0.01 (Pink, greenish spots)	0.35, 0.30, 0.25 (Dark violet spots)
Proteins and amino acids	Ninhydrin reagent	B	-	-	-	-	0.75, 0.61, 0.50, 0.32, 0.01 (Dark pink, violet spots)	0.70, 0.52, 0.35, 0.30, 0.25, 0.10 (Pink-reddish spots)

A: Ethyl Acetate: Methanol: Water (10:1.35:1.00); B: n-Butanol: Glacial acetic acid: Water (6:2:2)

P- Petroleum ether extract
C- Chloroform extract
M- Methanol extract
+- Present
B- Benzene extract
E- Ethyl acetate extract
W- Water extract
- - Absence

Table 3.7: Yield (% of methanol extract) obtained by fractionating methanol extract of selected drugs into different fractions.

Fraction/ Solvent used	Yield (% of methanol extract)		
	S. indicus	***C. pareira***	***C. orchioides***
Hexane	-	-	0.95
Petroleum ether	15.25	-	-
Benzene	18.75	-	-
Chloroform	5.00	-	1.98
Ethyl acetate	-	-	5.28
Methanol	58.00	-	88.50
Alkaloidal fraction	-	15.56	-
Non alkaloidal fraction	-	84.44	-

3.3 BIOLOGICAL SCREENING:

3.3.1 Immunomodulatory activity in normal animals:

3.3.1.1 Humoral response to SRBC (Haemagglutination antibody titre)

The humoral antibody titre value in control was found to be 64.00 ± 14.31. Administration of methanol extract and its fractions produced increase in humoral antibody titre as evident by haemagglutination at that dilution. Statistically significant levels were obtained with methanol extract and its petroleum ether, chloroform and remaining methanol fraction at both the dose level. The dose dependent increase was established only with petroleum ether, 256 ± 57.24($p < 0.001$) and 298.7 ± 71.39 ($p < 0.001$) at 100 and 200 mg/kg dose. Residual methanol fraction showed values of 341.3 ± 78.67 ($p<0.001$) and 426.7 ± 53.97 ($p<0.001$) at 100 and 200 mg/kg dose respectively. There was a decrease in antibody titre by methanol extract (682.7 ± 107.9 and 405.3 ± 69.46) and chloroform fraction (277.3 ± 76.92 and 160 ± 32) showed decrease in levels at higher dose. Benzene fraction showed increase in levels (170.7±75.12 and 138.7±25.69) but not statistically significant (Table 3.8, Fig 3.9).

Petroleum ether extract of *S. indicus* also tested for its effect on humoral antibody titre at five dose levels (50-400 mg/kg). It increases antibody titre in dose dependent manner initially but at higher doses levels reduced slightly. It showed antibody titre 140.80 ± 31.35, 256.00 ± 70.10 and 460.80 ± 76.80 ($p<0.01$) at doses 50, 100 and 200 mg/kg respectively. At doses 200 and 300 mg/kg levels decreased slightly, 307.20 ± 86.81 and 230.40 ±74.60 respectively (Table 3.9, Fig 3.10).

Water extract and its two fractions viz., n-butanol fraction and remaining aqueous fraction also tested for immunomodulatory activity at two dose levels (100 and 200 mg/kg). Water extract showed HA titre of 80.00 ± 16.00 and 112.00 ± 32.79, n-butanol fraction showed 69.33 ± 19.23 and 90.66 ± 36.40, whereas aqueous fraction showed, 64.00 ± 35.05 and 90.66 ± 17.36 respectively at 100 and 200 mg/kg. None of the tested

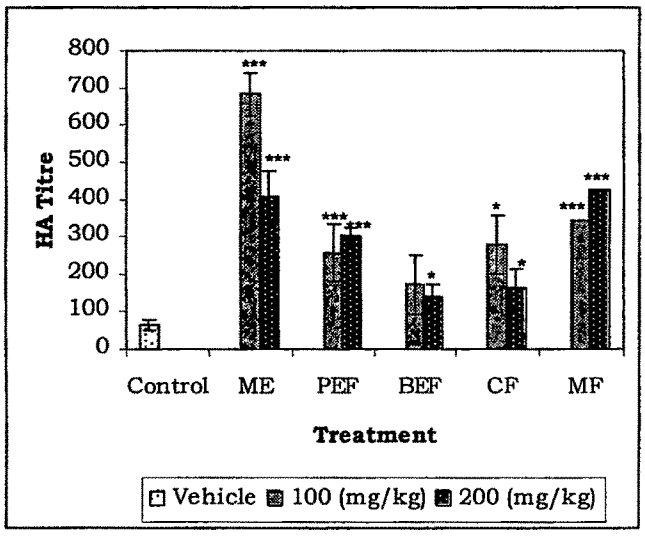


Fig 3.9: Effect of methanol extract of *S. indicus* and its different fractions on HA titre.

ME: Methanol extract **PEF: Petroleum ether fraction**

BEF: Benzene fraction **CF: Chloroform fraction**

MF: Methanol fraction

Values are expressed as mean ± S.E.M. n = 6;

*p<0.05; **p<0.01; ***p<0.001

All treated groups are compared with control.

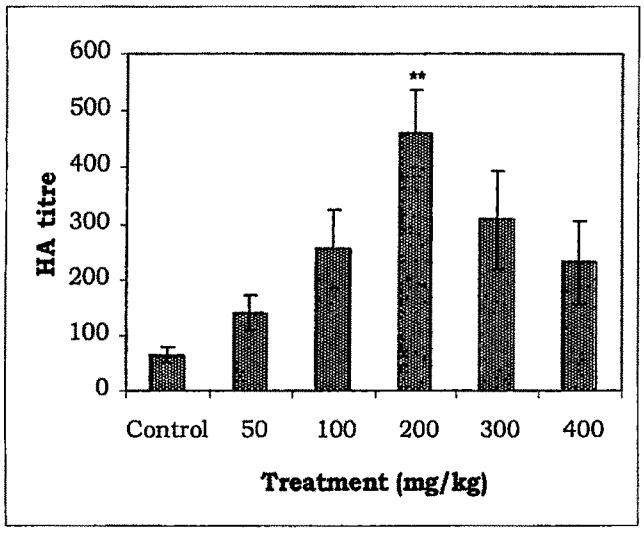


Fig 3.10: Effect of petroleum ether extract of *S. indicus* on HA titre.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

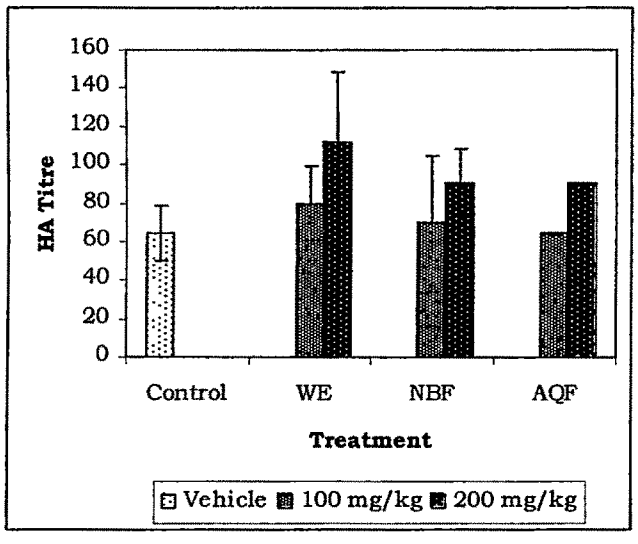


Fig 3.11: Effect of water extract of *S. indicus* and its fraction on HA titre.

WE: Water extract

NBF: n-Butanol fraction of water extract

AQF: Remaining aqueous fraction.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

extract/fraction could achieve statistically significant levels of HA titre when compared to control group (Table 3.10, Fig 3.11).

Methanol extract of the *C. pareira* was tested at five dose (50-800 mg/kg) levels for evaluating its effect on humoral response to sheep SRBC. The humoral antibody titre value in control was found to be 149.33 ± 35.70. Administration of methanol extract produced increase in humoral antibody titre as evident by haemagglutination at that dilution, but statistically significant increase couldn't obtained by any dose. Antibody titre at 50 mg/kg was 96.00 ± 14.11, 100 mg/kg was 128.00 ± 28.62, 200 mg/kg was 160.00 ± 32.00, 400 mg/kg was 181.33 ± 34.72 and 800 mg/kg 160.00 ± 32.00 (Table 3.11, Fig 3.12, A).

Alkaloidal fraction of *C. pareira* tested at four dose levels (25-100 mg/kg). Administration of alkaloidal fraction decreased level of antibody titre in dose dependent manner, however, higher doses had no effect on antibody titre compared to control. Alkaloidal fraction at 25 and 50 mg/kg, decreased levels significantly, 5.00 ± 1.00 ($p< 0.01$) and 8.00 ± 1.79 ($p< 0.01$) respectively. Further increase in dose was ineffective in decreasing the levels of antibody titre. Antibody titre at 75 and 100 mg/kg was 64.00 ± 14.31 (non significant) and 133 ± 40.83 respectively (Table 3.12, Fig 3.12, B).

Methanol extract of *C. orchioides* was screened at doses five doses ranging 50-800 mg/kg. Administration of methanol extract enhanced the antibody titre in dose dependent manner. However statistically significant increase could obtained at higher doses only. It showed levels of, 145.05 ± 40.20, 150.77 ± 35.04, 195.11 ± 25.22, 250.98 ± 11.20 ($p<0.01$) and 255.81 ± 18.90 ($p<0.01$) at doses 50, 100, 200, 400, 800 mg/kg, b.w. respectively (Table 3.13, Fig 3.13).

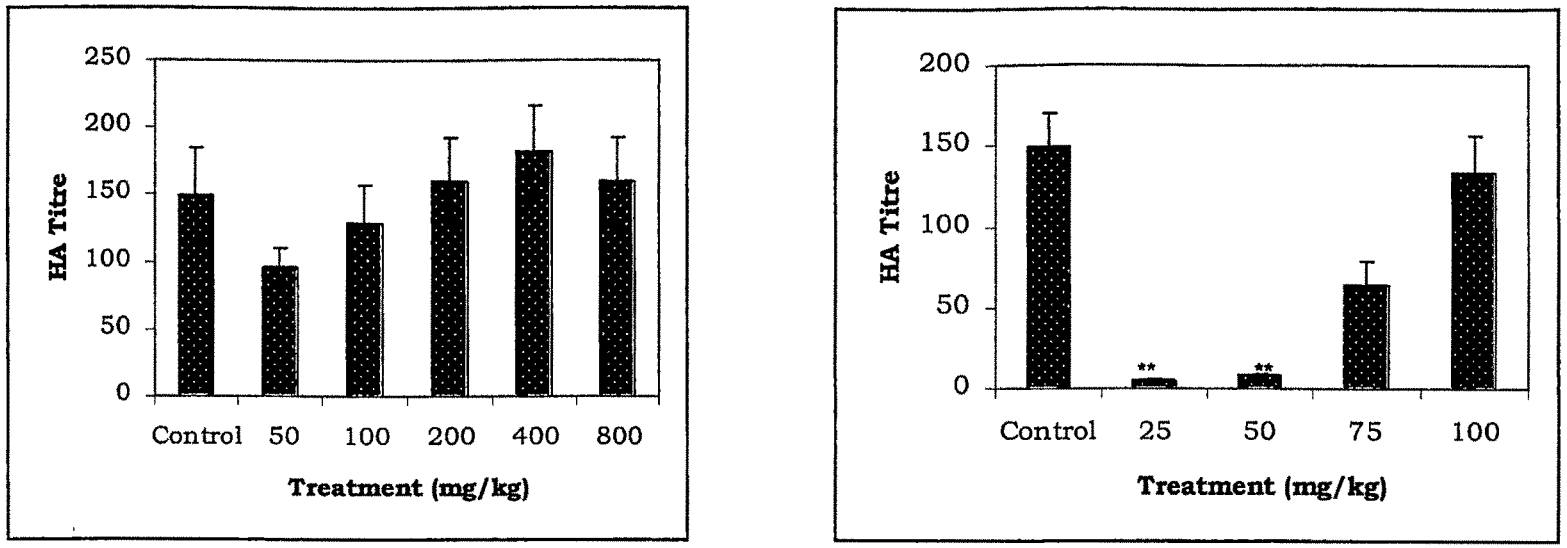


Fig 3.12: Effect of methanol extract (A) and alkaloidal fraction (B) of *C.pareira* on HA Titre.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

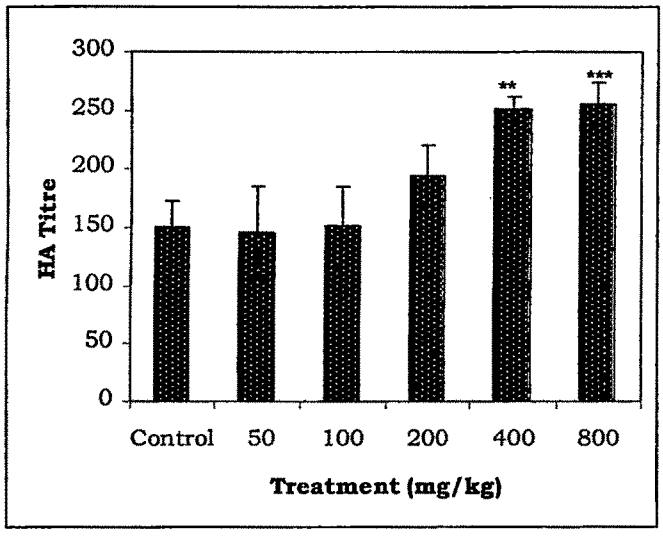


Fig 3.13: Effect of methanol extract of C. *orchioides* on HA titre.

Values are expressed as mean ± SEM.

Control group was compared with treated groups.

*p<0.05; **p<0.01; ***p<0.001.

3.3.1.2 Carbon clearance test

Methanol extract of *S.indicus* and its fractions in different solvents possess macrophage stimulatory activity as evidenced by increased phagocytic index in carbon clearance test. The phagocytic index for control group was found to be 0.094 ± 0.0065 whereas all test extracts of *S.indicus* increased it significantly. The maximum effect was found in case of methanol extract and its petroleum ether and remaining methanol fractions. Methanol extract showed phagocytic index, 0.150±0.008 ($p< 0.001$) and 0.126±0.005 ($p< 0.001$) at dose 100 and 200 mg/kg respectively. Petroleum ether fraction showed, 0.151±0.005 ($p<0.001$) and 0.167±0.004 ($p<0.001$) respectively at doses 100 and 200 mg/kg. Benzene fraction had phagocytic index of 0.125±0.002 and 0.123±0.005 ($p<0.001$), chloroform fraction showed, 0.123±0.003 and 0.139±0.005 ($p<0.001$), methanol fraction showed, 0.165±0.004 and 0.162±0.004 ($p<0.001$) at 100 and 200 mg/kg doses respectively. Methanol fraction and petroleum ether fraction showed highest phagocytic index of all extracts tested (Table 3.8, Fig 3.14).

Administration of petroleum ether extract of *S. indicus* at five dose levels resulted in dose dependent and linear increase in phagocytic index. Phagocytic indices obtained were as 0.09 ± 0.02, 0.12 ± 0.01, 0.13 ± 0.03, 0.15 ± 0.05 and 0.16 ± 0.02 at doses 50, 100, 200, 300, 400 mg/kg respectively (Table 3.9, Fig 3.15). However, statistically significant increase could obtain with 300 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.05$).

Water extract and its two fractions viz., n-butanol fraction and remaining aqueous fraction also screened for carbon clearance test at two dose levels (100 and 200 mg/kg). Water extract showed phagocytic index of 0.089 ± 0.004 and 0.097 ± 0.006, n-butanol fraction gave 0.121 ± 0.007 and 0.195 ± 0.030, whereas, aqueous fraction gave 0.091 ± 0.006 and 0.097 ± 0.005 respectively at 100 and 200 mg/kg. Only n-butanol fraction could raised the phagocytic index in statistically significant ($p<0.01$ and 0.001 at 100 and 200 mg/kg respectively) manner when compared to control group (Table 3.10; Fig 3.16).

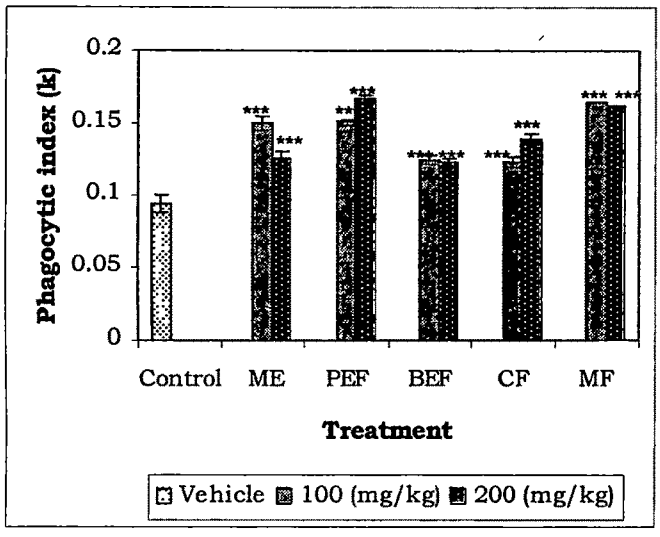


Fig 3.14: Effect of methanol extract and its different fractions of *S. indicus* on Phagocytic index (k).

ME: Methanol extract **PEF: Petroleum ether fraction**

BEF: Benzene fraction **CF: Chloroform fraction**

MF: Methanol fraction

Values are expressed as mean ± S.E.M. n = 6;

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

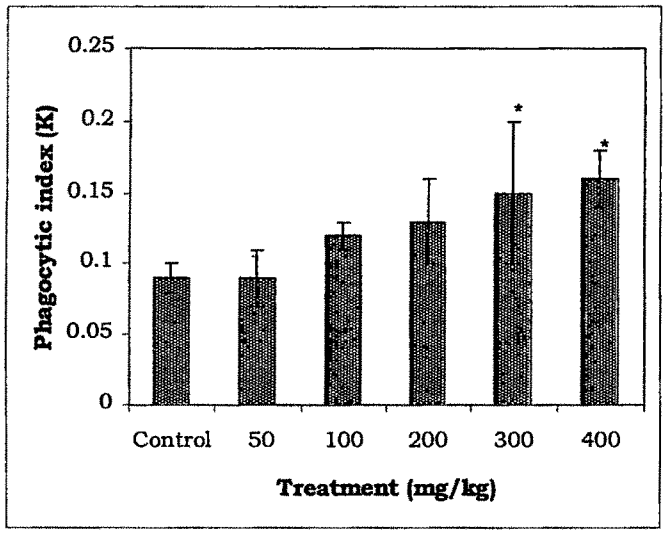


Fig 3.15: Effect of petroleum ether extract of *S. indicus* on phagocytic index.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

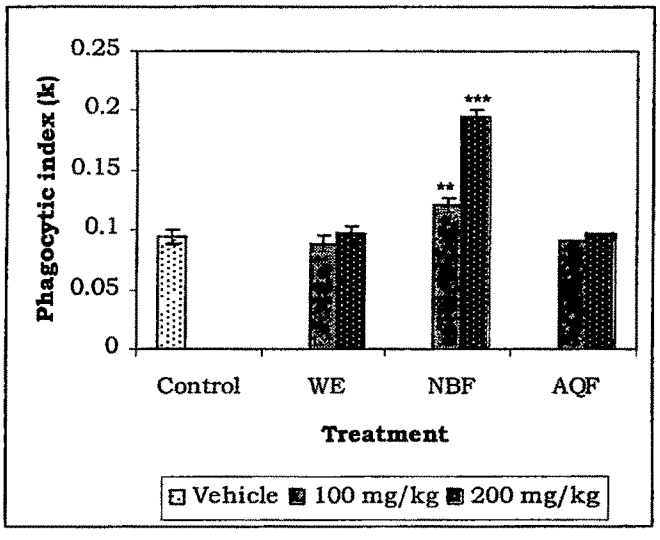


Fig 3.16: Effect of water extract of *S. indicus* and its fraction on Phagocytic index (k).

WE: Water extract

NBF: n-Butanol fraction of water extract

AQF: Remaining aqueous fraction.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

Methanol extract of *C. pareira* was tested at five doses for its effect on phagocytic index. It showed phagocytic index of 0.110 ± 0.001, 0.143 ± 0.012, 0.195 ± 0.009, 0.162 ± 0.002 and 0.187 ± 0.018 respectively at doses 50, 100, 200, 400 and 800 mg/kg. However, statistically significant increase could obtain at higher doses tested i.e. 200, 400 and 800 ($p < 0.001$) (Table 3.11, Fig 3.17 A).

Alkaloidal fraction of *C. pareira* was tested at four doses i.e. 25, 50, 75 and 100 mg/kg. It showed slight or no increase in phagocytic index at doses tested. At 25 and 50 mg/kg there was no increase, 0.102 ± 0.012 and 0.103 ± 0.009. At 75 and 100 mg/kg, there was a slight increase but statistically non significant, 0.112 ± 0.015 and 0.125 ± 0.020 (Table 3.12, Fig 3.17 B).

Methanol extract of *C. orchioides* was tested at five different dose levels (50-800 mg/kg). There was a dose dependent increase in phagocytic index on administration of methanol extract. It showed phagocytic index of, 0.108 ± 0.012, 0.138 ± 0.019 ($p<0.01$), 0.168 ± 0.009 ($p<0.01$), 0.202 ± 0.018 ($p<0.01$) and 0.212 ± 0.022 ($p<0.01$) at doses 50, 100, 200, 400 and 800 mg/kg, respectively (Table 3.13, Fig 3.18).

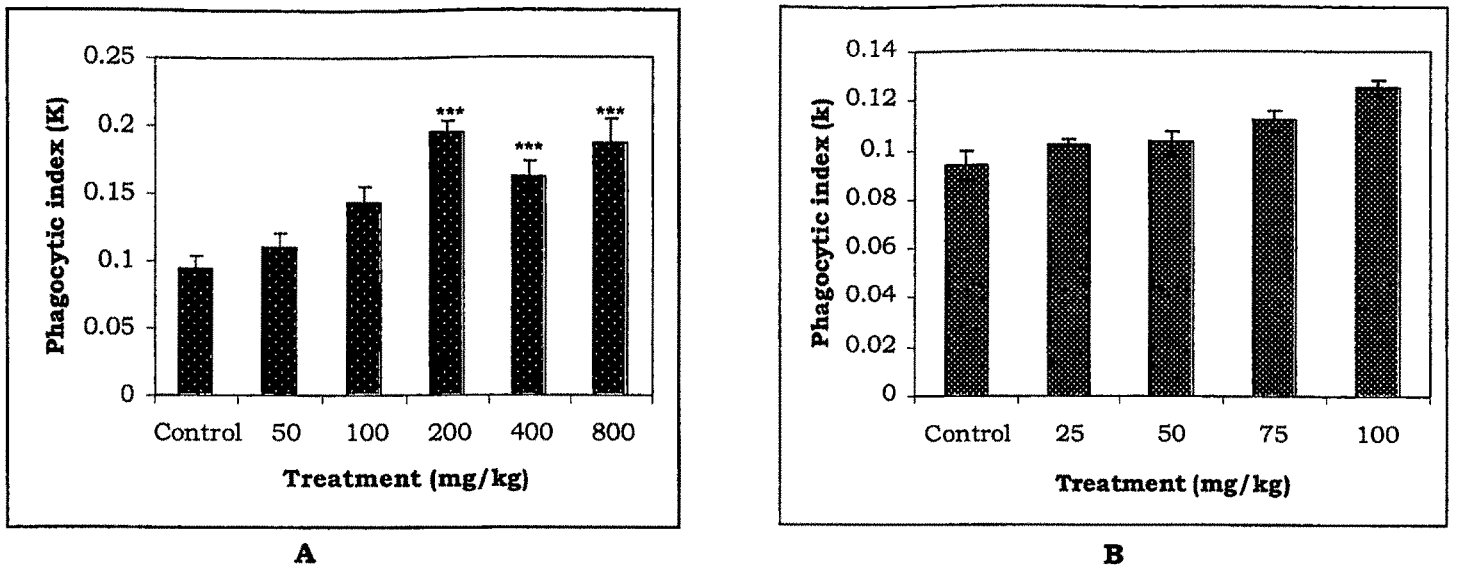


Fig 3.17: Effect of methanol extract (A) and alkaloidal fraction (B) of *C.pareira* on Phagocytic index (k).

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

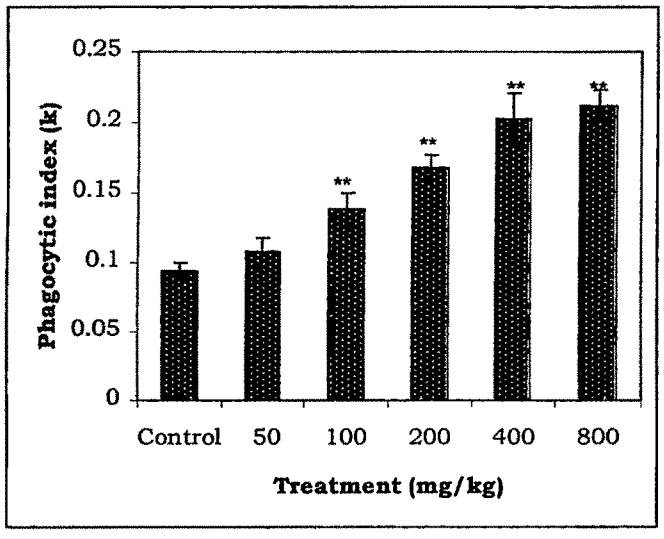


Fig 3.18: Effect of methanol extract of *C. orchioides* on Phagocytic index (k).

Values are expressed as mean ± SEM.

Control group was compared with treated groups.

*p<0.05; **p<0.01; ***p<0.001.

3.3.1.3 Delayed type hypersensitivity response

DTH response was checked by increased footpad thickness using digital vernier calipers. Administration of methanol extract of *S. indicus* and its fractions at doses 100 and 200 mg/kg, produced increase in thickness of footpad of mice as a measure of DTH response. Statistically significant ($p<0.001$) increase in DTH response in dose dependent manner by remaining methanol fraction (0.670 ± 0.050 and 0.810 ± 0.037) and petroleum ether fraction (0.470 ± 0.050 and 0.510 ± 0.037) obtained. Benzene fraction slightly reduced the DTH response (0.275 ± 0.033 and 0.246 ± 0.062) by reducing thickness of footpad compared to control group. Methanol extract (0.590 ± 0.054 and 0.576 ± 0.084) and chloroform fraction (0.521 ± 0.022 and 0.332 ± 0.059) also showed increased DTH response compared to control group, however at higher dose response declined (Table 3.8, Fig 3.19).

Administration of petroleum ether extract of *S. indicus* has resulted in stimulatory as well as suppressive effect depending on the doses. Lower doses 50 mg/kg and 100 mg/kg doses resulted in enhanced DTH response 0.51 ± 0.03 and 0.64 ± 0.05 respectively. Further increase in dose resulted in decreased DTH response 0.30 ± 0.03, 0.19 ± 0.02 and 0.17 ± 0.03 at 200, 300 and 400 mg/kg respectively (Table 3.9, Fig 3.20).

Administration of water extract of *S. indicus* and its fractions, n-butanol fraction and aqueous fraction resulted in DTH response 0.29 ± 0.005 and 0.31 ± 0.002, 0.37 ± 0.001 and 0.35 ± 0.008, 0.21 ± 0.010 and 0.25 ± 0.020 respectively at 100 and 200 mg/kg doses. None of the tested extract/fraction was found to statistically significant effect when compared to control group (Table 3.10, Fig 3.21).

Methanol extract of *C. pareira* was tested at five different doses (50-800 mg/kg) when administered orally showed a linear dose dependent increase in DTH response up to 400 mg/kg, however statistically significant increase could obtained at 200 mg/kg (1.00 ± 0.06) and 400 mg/kg (1.31 ± 0.17) doses ($p<0.01$ and $p<0.001$ respectively). At 800 mg/kg DTH response

was slightly reduced (0.91 ± 0.10). DTH response at doses 50 and 100 mg/kg was 0.46 ± 0.07 and 0.76 ± 0.08, respectively (Table 3.11, Fig 3.22 A).

Alkaloidal fraction of *C. pareira* was tested at four doses for its effect on DTH response (25-100 mg/kg). Administration of alkaloidal fraction resulted in decrease in DTH response in dose dependent manner. DTH response was found 0.31 ± 0.01, 0.23 ± 0.02, 0.15 ± 0.04 and 0.27 ± 0.02 at doses 25, 50, 75 and 100 mg/kg, respectively. However, statistically significant suppressive effect on DTH could obtained only with 75 mg/kg ($p<0.01$) (Table 3.12, Fig 3.22 B).

Administration of methanol extract of *C. orchioides* at five different doses resulted in increase in DTH response as evidenced by increase in paw thickness. Increase in DTH response was linear and dose dependent. It showed DTH response as 0.40 ± 0.01, 0.54 ± 0.01 ($p<0.01$), 0.94 ± 0.12 ($p<0.001$), 1.21 ± 0.14 ($p<0.001$) and 0.84 ± 0.15 ($p<0.001$) at doses 50, 100, 200, 400 and 800 mg/kg (Table 3.13, Fig 3.23).

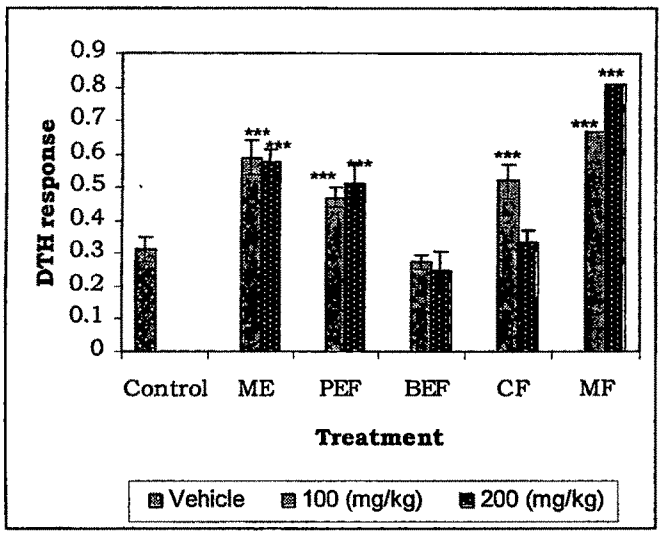


Fig 3.19: Effect of methanol extract and its different fractions of *S. indicus* on DTH response.

ME: Methanol extract **PEF: Petroleum ether fraction**
BEF: Benzene fraction **CF: Chloroform fraction**
MF: Methanol fraction

Values are expressed as mean ± S.E.M. n = 6;
*p<0.05; **p<0.01; ***p<0.001
All treated groups are compared with control.

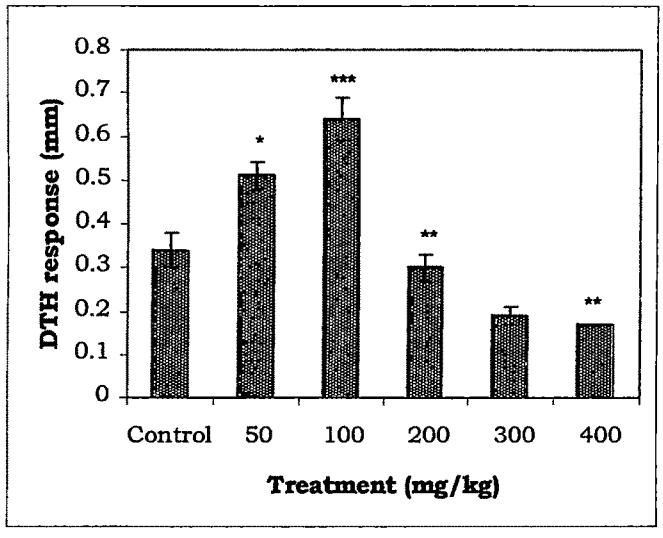


Fig 3.20: Effect of petroleum ether extract of *S. indicus* on DTH response.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

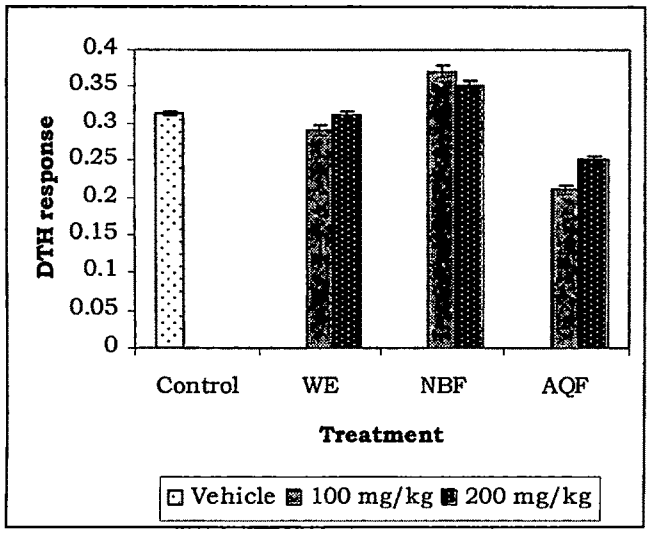


Fig 3.21: Effect of water extract of *S. indicus* and its fraction on DTH response.

WE: Water extract

NBF: n-Butanol fraction of water extract

AQF: Remaining aqueous fraction.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

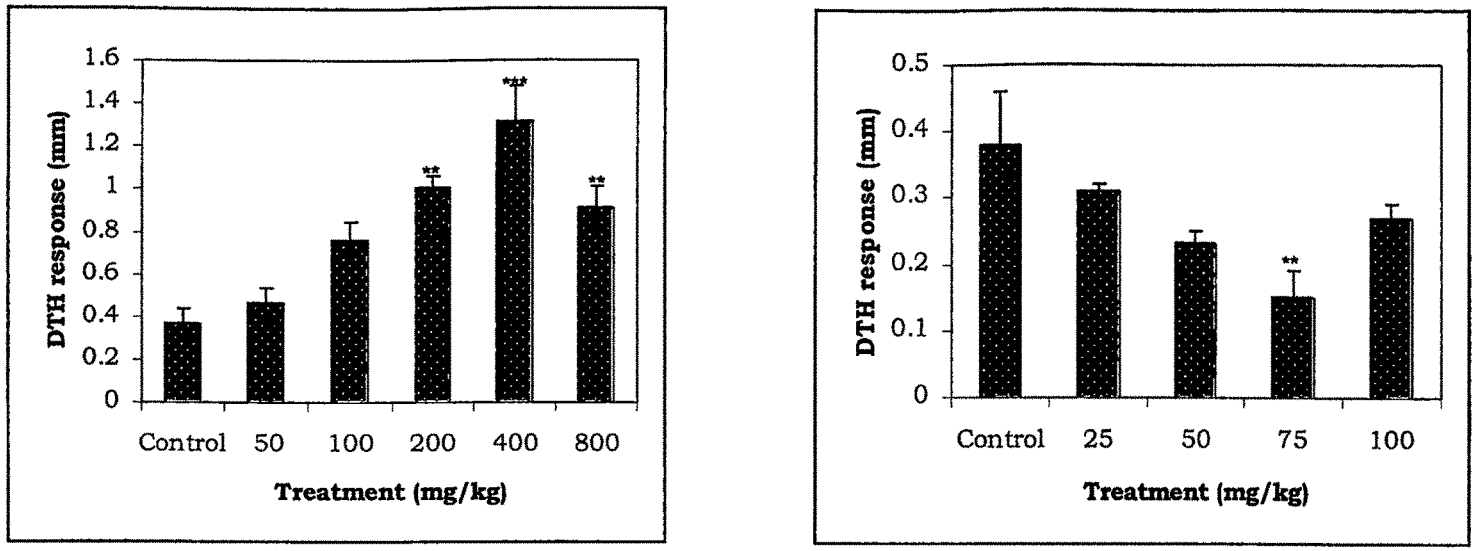


Fig 3.22: Effect of methanol extract (A) and alkaloidal fraction (AFCP) (B) of *C.pareira* on DTH response.

Values are expressed as mean ± SEM.

All treated groups are compared with control.

*p<0.05; **p<0.01; ***p<0.001

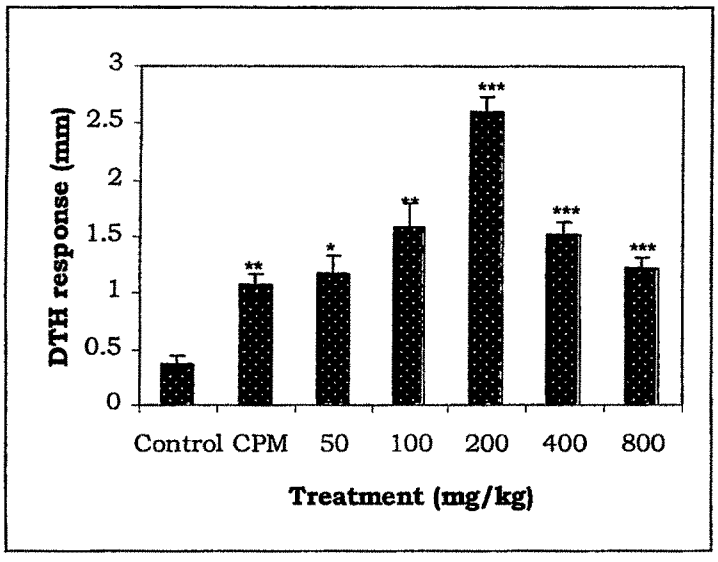


Fig 3.23: Effect of methanol extract of *C. orchioides* on DTH response.

Values are expressed as mean ± SEM.

Control group was compared with treated groups.

*p<0.05; **p<0.01; ***p<0.001.

3.3.1.4 Cyclophosphamide induced myelosuppression assay

Administration of cyclophosphamide has significantly lowered the levels of total WBC (2142 ± 248.08) as compared to control group (5800 ± 99.16) in blood. Methanol extract of *S. indicus* and its different fractions tested at two dose levels against cyclophosphamide induced myelosuppression. Methanol extract (4191 ± 214 and 4300 ± 154 at 100 and 200 mg/kg respectively) and residual methanol fraction (5275 ± 453 and 6083 ± 568 at 100 and 200 mg/kg respectively) were found potent in protecting cyclophosphamide-induced myelosuppression as evidenced by increasing the levels of total WBC count in dose dependent manner significantly ($p< 0.001$). Petroleum ether (3483 ± 222 and 3625 ± 197 100 and 200 mg/kg respectively) also raised WBC levels but could not reach up to the normal values. Benzene (2650 ± 172 and 3058 ± 157) and chloroform (2868 ± 101 and 3100 ± 96) slightly raised the levels (Table 3.8, Fig 3.24).

Water extract of *S. indicus* and its two fractions viz., n-butanol fraction and aqueous fraction, on simultaneous administration with cyclophosphamide showed WBC levels of 3150 ± 213.40 and 3215 ± 420.30, 3350 ± 205.80 and 3510 ± 115.90, 2900 ± 415.00 and 3050 ± 219.40 respectively at 100 and 200 mg/kg doses. Results clearly indicated that neither water extract nor its fractions offered any protection against cyclophosphamide induced myelosuppression (Table 3.10, Fig 3.25).

Administration of methanol extract of *C. pareira* at five different doses i.e. 50-800 mg/kg to mice simultaneously with cyclophosphamide resulted in increased WBC levels compared to cyclophosphamide alone. It showed WBC levels as 3408.30 ± 141, 4016.70 ± 270, 4575.00 ± 331, 6633.30 ± 421 and 7883.30 ± 257 at doses 50, 100, 200, 400 and 800 mg/kg respectively. However, statistically significant increase could obtain with higher doses tested, i.e. 400 mg/kg ($p<0.001$) and 800 mg/kg (0.01) (Table 3.11, Fig 3.26).

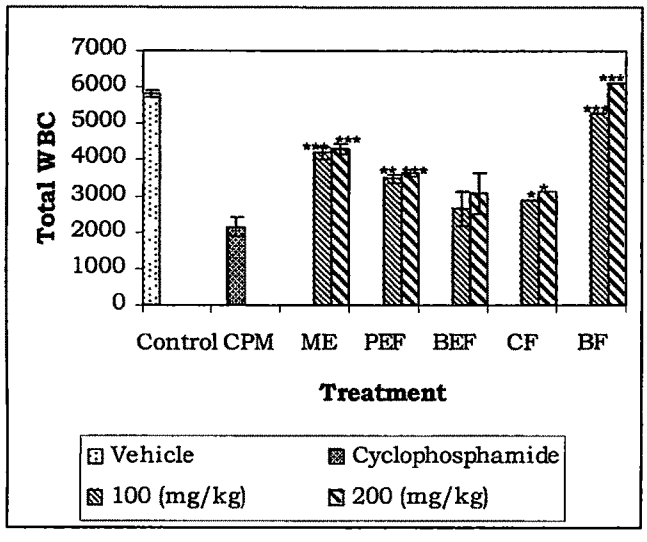


Fig 3.24: Effect of methanol extract and its different fractions of *S. indicus* on cyclophosphamide induced myelosuppression (Total WBC).

ME: Methanol extract **PEF: Petroleum ether fraction**

BEF: Benzene fraction **CF: Chloroform fraction**

MF: Methanol fraction **CPM: Cyclophosphamide**

Values are expressed as mean ± S.E.M. n = 6;

*p<0.05; **p<0.01; ***p<0.001

All treated groups with CPM.

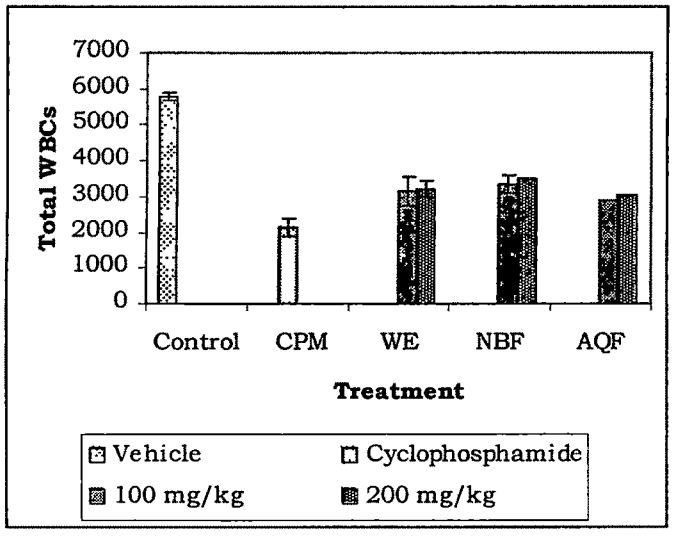


Fig 3.25: Effect of water extract of *S. indicus* and its fraction on cyclophosphamide induced myelosuppression assay (Total WBCs).

WE: Water extract

NBF: n-Butanol fraction of water extract

AQF: Remaining aqueous fraction.

Values are expressed as mean ± SEM.

Control group was compared with CPM group and all treated groups were compared with CPM group.

*p<0.05; **p<0.01; ***p<0.001

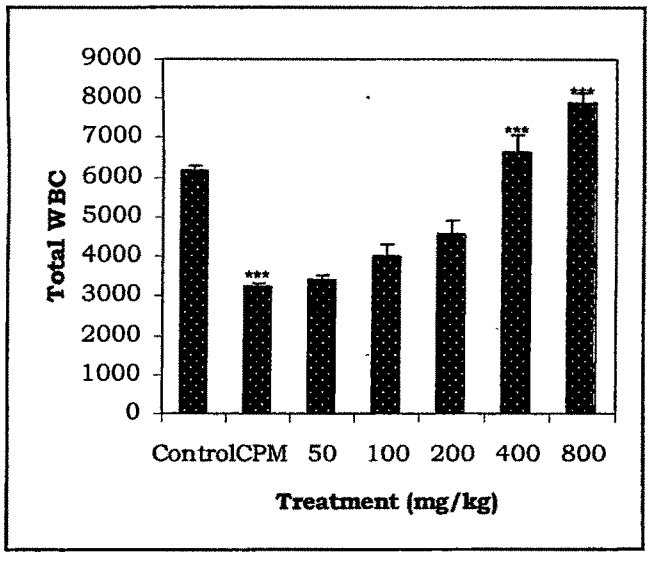


Fig 3.26: Effect of methanol extract of *C. pareira* on cyclophosphamide induced myelosuppression assay (Total WBCs).

Values are expressed as mean ± SEM.

Control group was compared with CPM group and all treated groups were compared with CPM group.

*p<0.05; **p<0.01; ***p<0.001

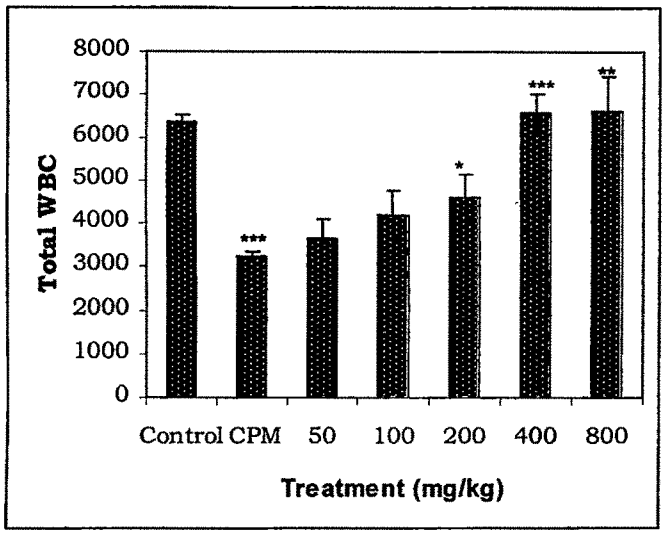


Fig 3.27: Effect of methanol extract of *C. orchioides* on cyclophosphamide induced myelosuppression (Total WBC).

n = six mice per group.

Control group was compared with CPM group and all treated groups were compared with CPM group.

Values are expressed as mean ± SEM.

*p<0.05; **p<0.01; ***p<0.001.

Administration of methanol extract of *C. orchioides* at five different doses i.e. 50-800 mg/kg to mice simultaneously with cyclophosphamide resulted in increased WBC levels compared to cyclophosphamide alone. It showed WBC levels as 3625.00 ± 455, 4183.30 ± 554, 4591.70 ± 537, 6550.00 ± 441 and 6583.30 ± 848 at doses 50, 100, 200, 400 and 800 mg/kg respectively. However, statistically significant increase could obtain with higher doses tested, i.e. 200 mg/kg ($p<0.05$), 400 mg/kg ($p<0.001$) and 800 mg/kg (0.01) (Table 3.13, Fig 3.27).

Table 3.8: Effect of methanol extract and its different fractions of *S. indicus* on HA titre, DTH response, phagocytic index and cyclophosphamide induced myelosuppression.

Treatment	Dose mg/kg	HA titre Mean ± S.E.M.	DTH response (mm) Mean paw edema ± S.E.M.	Phagocytic index Mean ± S.E.M.	Total WBC count Mean ± S.E.M.
Control	-	64.00 ± 14.311	0.313 ± 0.038	0.0943±0.006	5800±99.16
Cyclophosphamide	30	-	-	-	2142±248.1***
Methanol extract	100	682.7±107.9***	0.590 ± 0.054	0.150±0.008***	4191±214.6***
	200	405.3±69.46*	0.576 ± 0.084	0.126±0.005*	4300±154.4***
Petroleum ether fraction	100	256.0±57.24 N.S.	0.470 ± 0.050	0.151±0.005***	3483±222.4*
	200	298.7±71.39 N.S.	0.510 ± 0.037	0.167±0.004***	3625±197.8**
Benzene fraction	100	170.7±75.12 N.S.	0.275 ± 0.033	0.125±0.002**	2650±172.7 N.S.
	200	138.7±25.69 N.S.	0.246 ± 0.062	0.123±0.005*	3058±157.3 N.S.
Chloroform fraction	100	277.3±76.92 N.S.	0.521 ± 0.022	0.123±0.003*	2868±101.4 N.S.
	200	160.0±32.00 N.S.	0.332 ± 0.059	0.139±0.005***	3100±96.61 N.S.
Methanol fraction	100	341.3±78.67*	0.670 ± 0.050	0.165±0.004***	5275±453.1***
	200	426.7±53.97*	0.810 ± 0.037	0.162±0.004***	6083±568.9***
F value		6.851	12.485	19.304	23.349
P value		<0.0001	<0.0001	<0.0001	<0.0001

Values are expressed as mean ± S.E.M. n = 6;

*p< 0.05, **p<0.01, ***p<0.001, N.S. (Non Significant)

All treated groups are compared with control for HA titre, DTH response and phagocytic index, whereas with cyclophosphamide group in case of cyclophosphamide induced myelosuppression.

Table 3.9: Effect of petroleum ether extract of *S.indicus* on phagocytic index, HA titre and DTH response.

Group	Treatment	Dose (mg/kg)	Phagocytic index Mean ± S.E.M.	HA titre Mean ± S.E.M.	DTH response(mm) Mean paw edema ± S.E.M.
I	Control	-	0.09 ± 0.01	64.00 ± 14.311	0.34 ± 0.04
II	Pet.Ether Ext.	50	0.09 ± 0.02 ^{NS}	140.80 ± 31.35 ^{NS}	0.51 ± 0.03*
III	Pet.Ether Ext.	100	0.12 ± 0.01 ^{NS}	256.00 ± 70.10 ^{NS}	0.64 ± 0.05***
IV	Pet.Ether Ext.	200	0.13 ± 0.03 ^{NS}	460.80 ± 76.80**	0.30 ± 0.03 ^{NS}
V	Pet.Ether Ext.	300	0.15 ± 0.05*	307.20 ± 86.81 ^{NS}	0.19 ± 0.02 ^{NS}
VI	Pet.Ether Ext.	400	0.16 ± 0.02*	230.40 ±74.60 ^{NS}	0.17 ± 0.03**
F value			4.13	5.34	27.85
P value			<0.0076	<0.01	<0.0001

n = 6 mice per group,

Values are expressed as mean ± SEM.

Group I was compared with groups II, III, IV, V and VI.

*p<0.05; **p<0.01; ***p<0.001; $^{N.S.}$ = Non Significant

Table 3.10: Effect of water extract of *S. indicus* and its fractions on phagocytic index, DTH response and HA titre and cyclophosphamide induced myelosuppression (Total WBCs).

Groups	Treatment	Dose mg/kg	Phagocytic index (Mean ± S.E.M.)	DTH response (mm) (Mean ± S.E.M.)	HA titre (Mean ± S.E.M.)	Total WBC (mm^3) (Mean ± S.E.M.)
I	Control	-	0.0943 ± 0.006	0.313 ± 0.038	64.00 ± 14.311	5800 ± 99.16
II	CPM	50	-	-	-	2142 ± 248.1
III	Water Ext.	100	0.089 ± 0.004	0.29 ± 0.005	80.00 ± 16.00	3150 ± 213.40
IV	Water Ext.	200	0.097 ± 0.006	0.31 ± 0.002	112.00 ± 32.79	3215 ± 420.30
V	n-butanol fraction	100	0.121 ± 0.007 **	0.37 ± 0.001	69.33 ± 19.23	3350 ± 205.80
VI	n-butanol fraction	200	0.195 ± 0.030 ***	0.35 ± 0.008	90.66 ± 36.40	3510 ± 115.90
VII	Water fraction	100	0.091 ± 0.006	0.21 ± 0.010	64.00 ± 35.05	2900 ± 415.00
VIII	Water fraction	200	0.097 ± 0.005	0.25 ± 0.020	90.66 ± 17.36	3050 ± 219.40
F Value			8.96	1.29	0.37	2.35
P value			0.0001	N.S.	N.S.	N.S.

n = 6 mice per group, Values are expressed as mean ± SEM.

Group I compared with III, IV, V, VI and VII for HA titre, DTH response and phagocytic index.

Group I compared with II and Group II compared with III, IV, V, VI, VII and VIII for cyclophosphamide induced myelosuppression assay.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

Table 3.11: Effect of methanol extract of *C.pareira* on phagocytic index, DTH response and HA titre and cyclophosphamide induced myelosuppression (Total WBCs).

Groups	Treatment	Dose (mg/kg)	Phagocytic index (Mean ± S.E.M.)	DTH response (mm) (Mean ± S.E.M.)	HA titre (Mean ± S.E.M.)	Total WBC (mm^3) (Mean ± S.E.M.)
I	Control	-	0.094 ± 0.001	0.37 ± 0.07	149.33 ± 35.70	6175.00 ± 98.11
II	CPM		-	-	-	3250.00 ± 73.03***
III	Methanol extract	50	0.110 ± 0.001 N.S.	0.46 ± 0.07 N.S.	96.00 ± 14.11 N S	3408.30 ± 141.00 NS
IV	Methanol extract	100	0.143 ± 0.012 N.S.	0.76 ± 0.08 N.S.	128.00 ± 28.62 N.S	4016.70 ± 270.08 NS
V	Methanol extract	200	0.195 ± 0.009***	1.00 ± 0.06**	160.00 ± 32.00 N.S	4575.00 ± 331.16 NS
VI	Methanol extract	400	0.162 ± 0.012***	1.31 ± 0.17***	181.33 ± 34.72 N.S	6633.30 ± 421.24***
VII	Methanol extract	800	0.187 ± 0.018***	0.91 ± 0.10**	160.00 ± 32.00 N.S	7883.30 ± 257.05***
F Value			12.10	12.41	2.06	32.63
P value			<0.0001	<0.0001	Non significant	<0.0001

n = 6 mice per group, Values are expressed as mean ± SEM.

Group I compared with III, IV, V, VI and VII for HA titre, DTH response and phagocytic index.

Group I compared with II and Group II compared with III, IV, V, VI and VII for cyclophosphamide induced myelosuppression assay.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

Table 3.12: Effect of alkaloidal fraction of *C. pareira* on phagocytic index, HA titre and DTH response.

Treatment	Dose (mg/kg)	Phagocytic index (k) (Mean ± S.E.M.)	HA Titre (Mean ± S.E.M.)	DTH (Mean ± S.E.M.)
Control	-	0.094 ± 0.0065	149.33 ± 35.70	0.38 ± 0.08
Alkaloidal fraction	25	0.102 ± 0.012	5.00 ± 1.00**	0.31 ± 0.01 N.S.
Alkaloidal fraction	50	0.103 ± 0.009	8.00 ± 1.79**	0.23 ± 0.02 N.S.
Alkaloidal fraction	75	0.112 ± 0.015	64.00 ± 14.31N.S.	0.15 ± 0.04**
Alkaloidal fraction	100	0.125 ± 0.020	133.33 ± 40.83 N.S.	0.27 ± 0.02 N.S.
F value		2.50	7.30	4.01
P value		Non significant	<0.0005	<0.012

n = 6 mice per group,

Values are expressed as mean ± SEM.

Control group was compared with treated groups.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

Table 3.13: Effect of methanol extract of *C. orchioides* on phagocytic index, DTH response and HA titre and cyclophosphamide induced myelosuppression (Total WBCs).

Groups	Treatment	Dose (mg/kg)	Phagocytic index (Mean ± S.E.M.)	DTH response (mm) (Mean ± S.E.M.)	HA titre (Mean ± S.E.M.)	Total WBC (mm^3) (Mean ± S.E.M.)
I	Control	-	0.094 ± 0.001	0.37 ± 0.07	149.33 ± 35.70	6175.00 ± 98.11
II	CPM		-	-	-	3250.00 ± 73.03***
III	Methanol extract	50	0.108 ± 0.012	0.40 ± 0.01 N.S.	145.05 ± 40.20	3625.00 ± 455 N.S.
IV	Methanol extract	100	0.138 ± 0.019**	0.54± 0.01**	150.77 ± 35.04	4183.30 ± 554 N.S.
V	Methanol extract	200	0.168 ± 0.009**	0.94 ± 0.12***	195.11 ± 25.22	4591.70 ± 537*
VI	Methanol extract	400	0.202 ± 0.018**	1.21 ± 0.14***	250.98 ± 11.20*	6550.00 ± 441***
VII	Methanol extract	800	0.212 ± 0.022**	0.84 ± 0.15***	255.81 ± 18.90*	6583.30 ± 848**
F Value			16.87	10.54	4.98	28.64
P value			<0.0001	<0.0001	<0.001	<0.0001

n = 6 mice per group, Values are expressed as mean ± SEM.

Group I compared with III, IV, V, VI and VII for HA titre, DTH response and phagocytic index.

Group I compared with II and Group II compared with III, IV, V, VI and VII for cyclophosphamide induced myelosuppression assay.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

3.3.2 Immunomodulatory activity in immunosuppressed animals:

3.3.2.1 Effect of test extracts and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in mice-7 days pretreatment.

Residual methanol fraction (Bioactive fraction) of *S. indicus*, methanol extract of *C. orchioides* and methanol extract of *C. pareira* were tested for their effect on immune system in immunosuppressed animals.

Administration of cyclophosphamide resulted in decreased HA titre i.e. 2.67 ± 000.42. The reduction was significant ($p<0.05$) when compared to control animals, which showed antibody titre of 64.00 ± 14.31. When compared with cyclophosphamide treated animals, animals receiving residual methanol fraction treatment along with cyclophosphamide (50-800 mg/kg) showed dose dependent recovery in HA titre. The values of antibody titre obtained at 50, 100, 200, 400 and 800 mg/kg doses were 3.00 ± 000.44, 56.00 ± 023.18, 512.00 ± 114.49, 1024.00 ± 228.97 and 192.00 ± 028.62 respectively. However, significant increase in HA titre could be obtained at 200 ($p<0.05$) and 400 mg/kg ($p<0.001$) dose. At higher dose it decreased (Table 3.14, Fig 3.28).

A significant increase in paw edema was observed in animals treated with methanol fraction on day 8 after challenge on day 7 with SRBCs. Animals treated with cyclophosphamide showed significant increase in DTH response ($p< 0.05$) compared to control animals. Cyclophosphamide showed DTH response of 0.590 ± 0.05., whereas control group showed DTH response 0.313 ± 0.04. An increase in the DTH response was observed on treatment of bioactive fraction and cyclophosphamide. Administration of methanol fraction at doses 50, 100, 200, 400, 800 mg/kg resulted in DTH responses of 0.576 ± 0.08, 0.670 ± 0.05, 0.810 ± 0.04, 0.875 ± 0.03 and 0.546 ± 0.06 respectively. However, significant increase in DTH, compared to cyclophosphamide treated group was noted only at 400 mg/kg ($p< 0.001$) (Table 3.14, Fig 3.28). Thus it can be observed that bioactive fraction of *S.indicus* acts as potentiator of DTH.

Administration of methanol extract of *C. pareira* along with cyclophosphamide resulted in increased levels of antibody titre as compared to group that received cyclophosphamide alone. Animals receiving different doses of extract viz., 50, 100, 200, 400 and 800 mg/kg showed antibody titre, 53.33 ± 6.75, 85.33 ± 13.49, 117.33 ± 30.54, 149.33 ± 35.70 and 96.00 ± 14.31. Antibody titre of control and cyclophosphamide group was found 149.33 ± 35.70 and 10.00 ± 2.683 respectively. Cyclophosphamide significantly lowered (0.01) the antibody titre when compared to group that received cyclophosphamide alone. Methanol extract of *C. pareira* afforded significant protection against cyclophosphamide only at 200 mg/kg (0.05) and 400 mg/kg (0.01).

Methanol extract of *C. pareira* was found strong potentiator of DTH response induced by cyclophosphamide. DTH responses recorded at doses 50, 100, 200, 400 and 800 mg/kg were as 1.075 ± 0.408, 1.210 ± 0.199, 1.250 ± 0.354, 1.850 ± 0.463 and 1.612 ± 0.509 respectively. DTH responses of control and cyclophosphamide group were 0.377 ± 0.078 and 1.070 ± 0.106 respectively. All treated groups showed statistically significant differences when compared to control group, whereas in comparison with cyclophosphamide group except 50 mg/kg, remaining groups showed significant change (Table 3.15, Fig 3.29).

Animals treated with different doses of methanol extract of *C. orchioides* and receiving cyclophosphamide showed increase in haemagglutination titre in dose dependent manner, as 74.68 ± 10.67, 149.33 ± 35.69, 192.00 ± 28.62, 256.00 ± 80.95 and 128.00 ± 28.62 at 50, 100, 200, 400 and 800 mg/kg doses respectively, compared to cyclophosphamide group which showed HA titre of 10.00 ± 2.68. Increase in haemagglutination antibody titre was found insignificant compared to control group (149.33 ± 35.70). However, compared to group receiving only cyclophosphamide it was found significant.

Animals treated with cyclophosphamide and receiving different doses of extract showed significant change in DTH response as compared to cyclophosphamide alone. DTH responses obtained at doses 50, 100, 200, 400 and 800 mg/kg were found, 1.17 ± 0.17, 1.58 ± 0.22, 2.60 ± 0.13, 1.51 ± 0.12 and 1.22 ± 0.10 respectively. DTH response of cyclophosphamide group was found 1.07 ± 0.10. Thus it can be observed that methanol extract of *C. orchioides* acts as potentiator of DTH (Table 3.16, Fig 3.30).

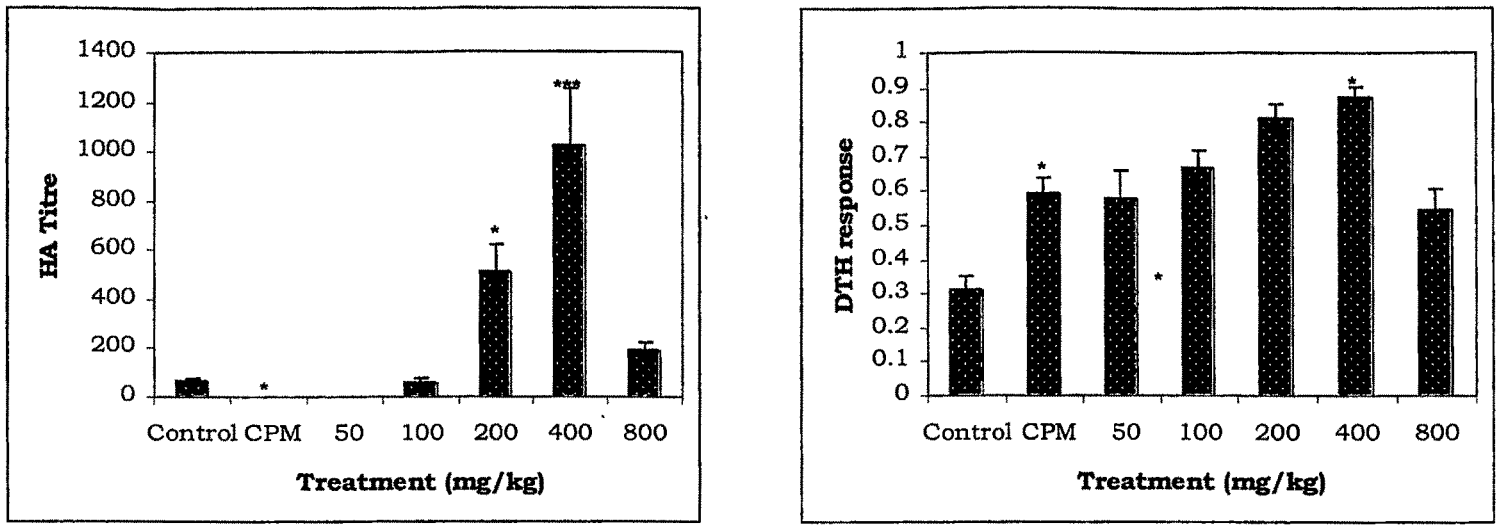


Fig 3.28: Effect of bioactive fraction of *S.indicus* and Cyclophosphamide (CPM) on HA titre and DTH response using SRBCs as antigen- 7 days pretreatment.

Values are expressed as mean ± SEM.

Group II was compared with group I.

Groups III, IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001.

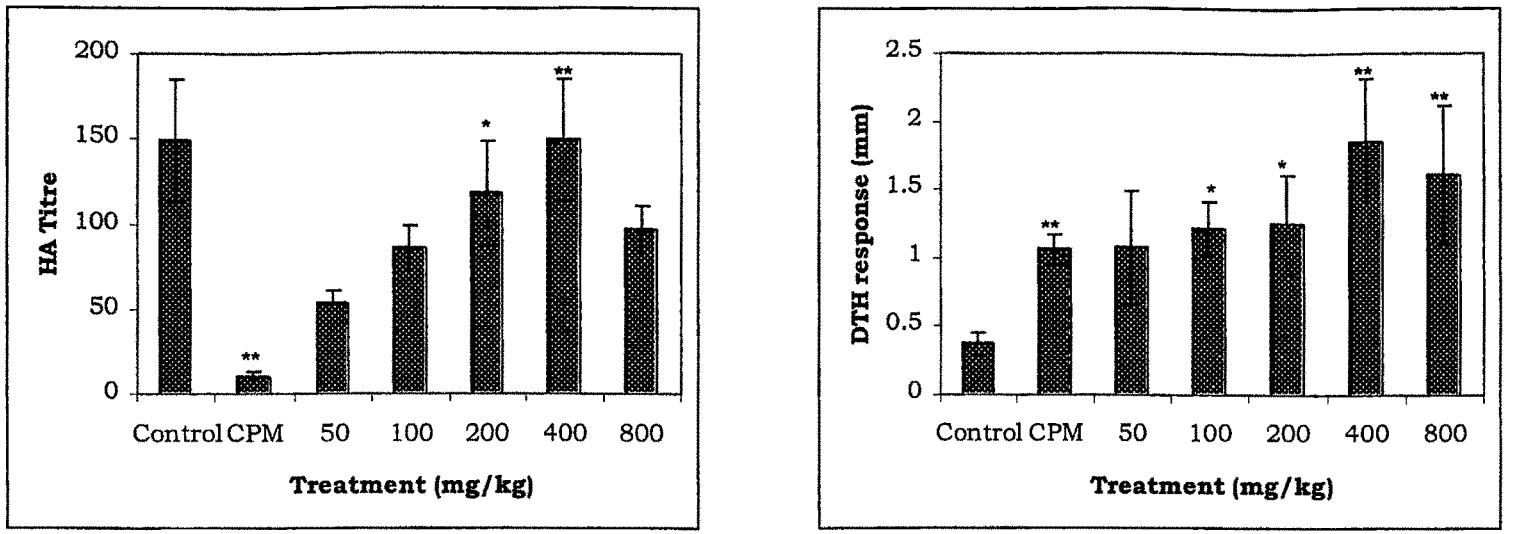


Fig 3.29: Effect of methanol extract of *C.pareira* and Cyclophosphamide (CPM) on HA titre and DTH response using SRBCs as antigen- 7 days pretreatment.

Values are expressed as mean ± SEM.

Group II was compared with group I.

Groups III, IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001.

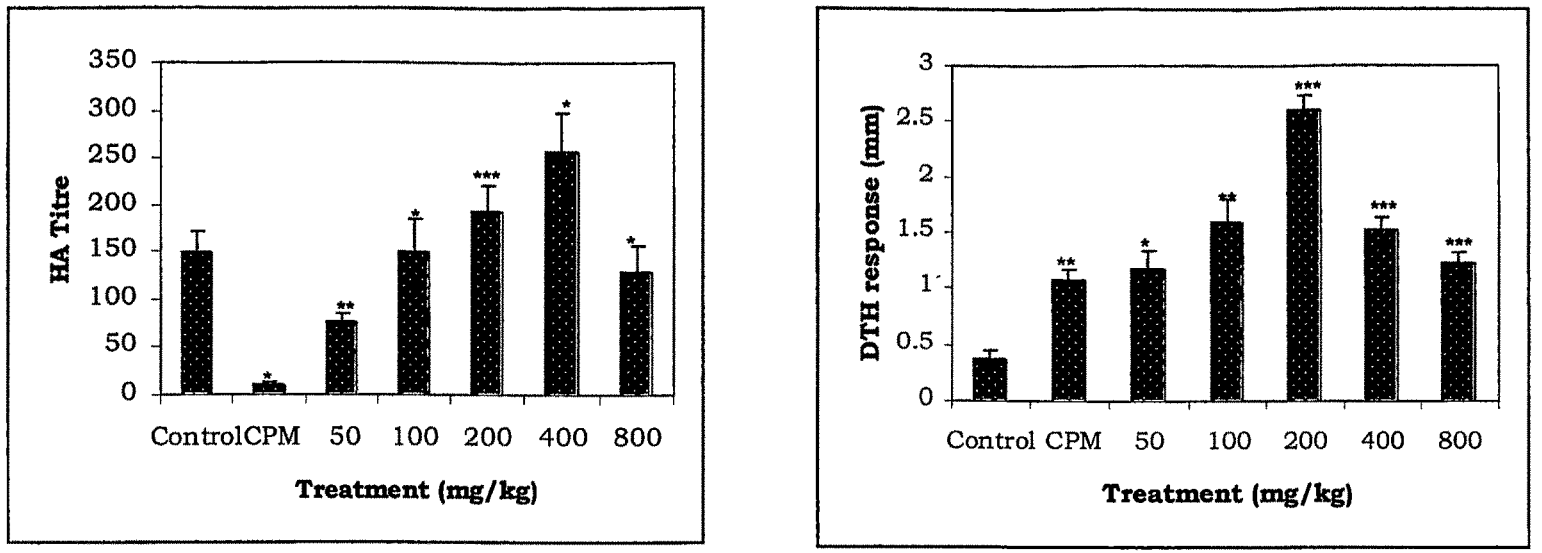


Fig 3.30: Effect of methanol extract of *C. orchioides* and Cyclophosphamide (CPM) on HA titre and DTH response using SRBCs as antigen- 7 days pretreatment.

Values are expressed as mean ± SEM.

Group II was compared with group I.

Groups III, IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001.

Table 3.14: Effect of bioactive fraction of *S.indicus* and Cyclophosphamide (CPM) on HA titre and DTH response using SRBCs as antigen- 7 days pretreatment.

Groups	Treatment	Dose (mg/kg)	HA titre Mean±S.E.M.	DTH response(mm) Mean paw edema ±S.E.M.
I	Control	-	64.00 ± 014.31	0.313 ± 0.04
II	CPM	50	2.67 ± 000.42*	0.590 ± 0.05 *
III	Bioactive fraction + CPM	50	3.00 ± 000.44 N.S.	0.576 ± 0.08 N.S.
IV	Bioactive fraction + CPM	100	56.00 ± 023.18 N.S.	0.670 ± 0.05 N.S.
V	Bioactive fraction + CPM	200	512.00 ± 114.49 *	0.810 ± 0.04 N.S.
VI	Bioactive fraction + CPM	400	1024.00 ± 228.97 ***	0.875 ± 0.03 *
VII	Bioactive fraction + CPM	800	192.00 ± 028.62 N.S.	0.546 ± 0.06 N.S.
F value			15.022	11.726
P value			0.0001	0.0001

n = 6 mice per group,

Values are expressed as mean ± SEM.

Group II was compared with group I.

Groups III, IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

Table 3.15: Effect of methanol extract of *C. pareira* and Cyclophosphamide (CPM) on HA titre and DTH response using SRBCs as antigen- 7 days pretreatment.

Group	Treatment	Dose (mg/kg)	HA titre Mean±S.E.M.	DTH response (mm) Mean paw edema ±S.E.M.
I	Control	-	149.33 ± 35.69	0.37 ± 0.07
II	CPM	50	10.00 ± 2.68*	1.07 ± 0.10*
III	MeOH Ext. + CPM	50	53.33 ± 6.75	1.07 ± 0.40
IV	MeOH Ext. + CPM	100	85.33 ± 13.49	1.21 ± 0.19
V	MeOH Ext. + CPM	200	117.33 ± 30.54*	1.25 ± 0.35
VI	MeOH Ext. + CPM	400	149.33 ± 35.69**	1.85 ± 0.46
VII	MeOH Ext. + CPM	800	96.00 ± 14.31	1.61 ± 0.50
F value			4.599	1.851
P value			<0.0015	<0.1176 (N.S.)

n = 6 mice per group,

Values are expressed as mean ± SEM.

Group II was compared with group I.

Groups III, IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

Table 3.16: Effect of methanol extract of *C. orchioides* and Cyclophosphamide (CPM) on HA titre and DTH response using SRBCs as antigen- 7 days pretreatment.

Group	Treatment	Dose (mg/kg)	HA titre Mean±S.E.M.	DTH response (mm) Mean paw edema ±S.E.M.
I	Control	-	149.33 ± 35.69	0.37 ± 0.07
II	CPM	50	10.00 ± 2.68*	1.07 ± 0.10*
III	MeOH Ext. + CPM	50	74.68 ± 10.67 N.S.	1.17 ± 0.17 N.S.
IV	MeOH Ext. + CPM	100	149.33 ± 35.69 N.S.	1.58 ± 0.22 N.S.
V	MeOH Ext. + CPM	200	192.00 ± 28.62*	2.60 ± 0.13***
VI	MeOH Ext. + CPM	400	256.00 ± 80.95**	1.51 ± 0.12 N.S.
VII	MeOH Ext. + CPM	800	128.00 ± 28.62 N.S.	1.22 ± 0.10 N.S.
F value			4.037	22.809
P value			<0.0035	<0.0001

n = 6 mice per group,

Values are expressed as mean ± SEM.

Group II was compared with group I.

Groups III, IV, V, VI and VII were compared with group II.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

3.3.2.2 Effect of test extracts on HA titre and DTH response using SRBCs as an antigen in mice-15 days pretreatment.

The humoral antibody titre value in control was found to be 64.00 ± 14.31. Administration of methanol fraction of *S. indicus* produced increase in humoral antibody titre as evident by haemagglutination at that dilution. The dose dependent increase was established with methanol fraction. Values of HA titre obtained at 50, 100, 200, 400 and 800 mg/kg dose were 298.67 ± 71.39, 384.00 ± 57.24, 640.00 ± 128.00, 853.33 ± 107.94 and 448.00 ± 131.16 respectively. Statistically significant increase was observed with 200 mg/kg ($p< 0.01$) and 400 mg/kg ($p< 0.001$). Activity declines at higher dose.

There was dose dependent increase in DTH response observed on administration with bioactive fraction of *S. indicus*, 0.370 ± 0.027, 0.388 ± 0.042, 0.520 ± 0.089, 0.456 ± 0.068 and 0.337 ± 0.052 at doses 50, 100, 200, 400 and 800 mg/kg respectively. However, statistical significant increase in paw edema when compared to control group was found at 200 mg/kg ($p< 0.01$) only (Table 3.17, Fig 3.31).

Administration of methanol extract of *C. pareira* for 15 days resulted in slight decrease in levels of antibody titre at lower doses. HA titre values obtained with doses 50, 100, 200, 400 and 800 mg/kg were 85.33 ± 13.49, 128.00 ± 28.02, 149.33 ± 35.70, 117.35 ± 74.82 and 106.67 ± 31.64 respectively. Antibody titre for control group was 149.33 ± 35.70. In short, administration of methanol extract resulted in suppression of humoral immunity.

DTH response was suppressed by lower doses of methanol extract of *C. pareira,* whereas higher doses increase the same. DTH responses for doses 50, 100, 200, 400 and 800 mg/kg were as 0.20 ± 0.05, 0.25 ± 0.04, 0.35 ± 0.01, 0.64 ± 0.12 and 0.82 ± 0.15 respectively. DTH response was significantly suppressed by 50 mg/kg (0.05) and 100 mg/kg (0.05), whereas it was significantly stimulated by 400 mg/kg (0.01) and 800 mg/kg (0.01) (Table 3.18, Fig 3.32).

In this 15 days treatment schedule effect of methanol extract of *C. orchioides* showed increasing levels of haemagglutination antibody titre. This increase follows in dose dependent manner up to 400 mg/kg, at higher dose i.e.800 mg/kg a decrease in level of antibody titre was noted. HA titre of groups receiving methanol extract of C. orchioides were found, 138.67 ± 25.69, 213.33 ± 26.98, 298.67 ± 71.39, 320.00 ± 64.00 and 170.67 ± 26.98 respectively at 50, 100, 200, 400 and 800 mg/kg dose. HA titre of control group was 149.33 ± 35.69. Significant increase compared to control was only noted with 400 mg/kg ($p<0.01$).

DTH response, which is direct, co-relate of cell mediated immunity was found to be significantly increased when methanol extract of *C. orchioides* administered at dose 50-200 mg/kg in dose dependent manner whereas at higher doses it declines. DTH responses recorded for 50, 100, 200, 400 and 800 mg/kg were as 0.52 ± 0.04, 0.85 ± 0.05, 1.09 ± 0.06, 0.87 ± 0.05 and 0.46 ± 0.05 respectively (Table III). Statistical significant response was obtained only with 100 mg/kg ($p<0.001$), 200 mg/kg ($p<0.0001$) and 400 mg/kg ($p<0.001$) (Table 3.19, Fig 3.33).

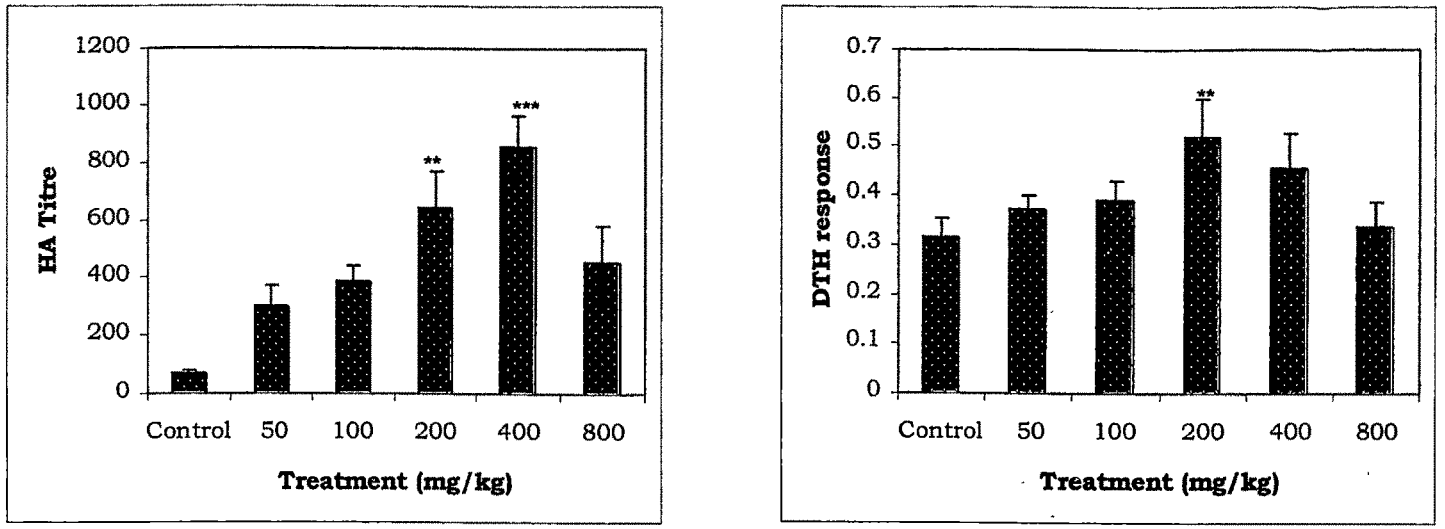


Fig 3.31: Effect of bioactive fraction of *S. indicus* on HA titre and DTH response using SRBCs as antigen- 15 days pretreatment.

Values are expressed as mean ± SEM.

Group I was compared with groups II, III, IV, V and VI.

*p<0.05; **p<0.01; ***p<0.001.

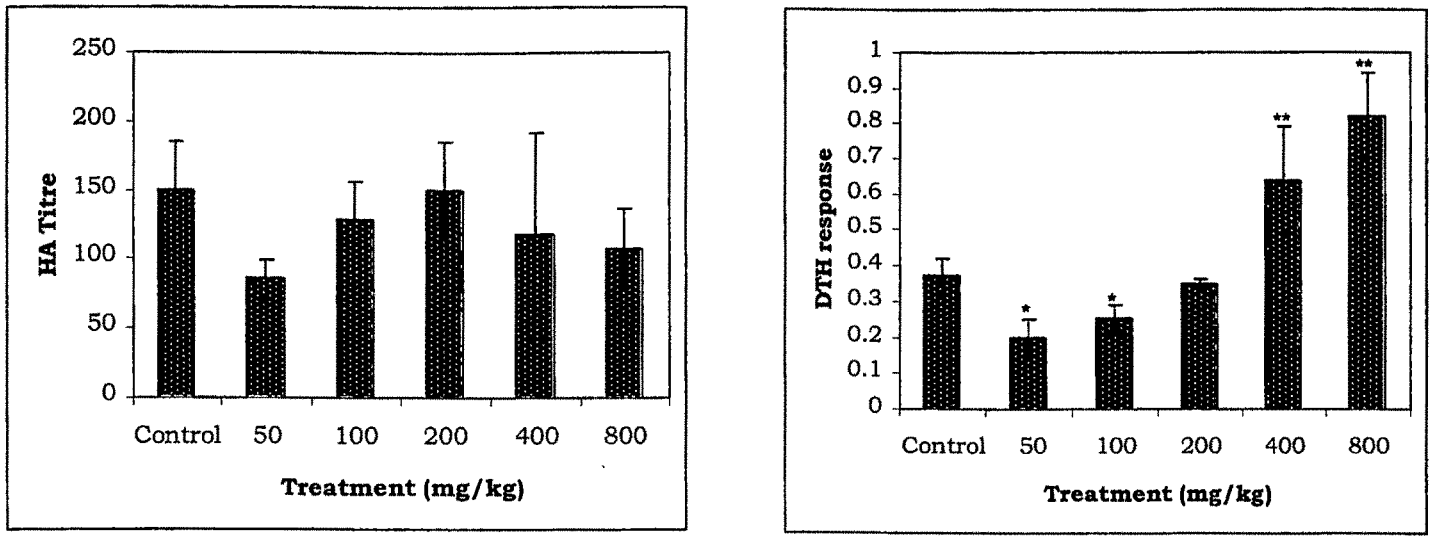


Fig 3.32: Effect of methanol extract of *C. pareira* on HA titre and DTH response using SRBCs as antigen- 15 days pretreatment.

Values are expressed as mean ± SEM.

Group I was compared with groups II, III, IV, V and VI.

*p<0.05; **p<0.01; ***p<0.001.

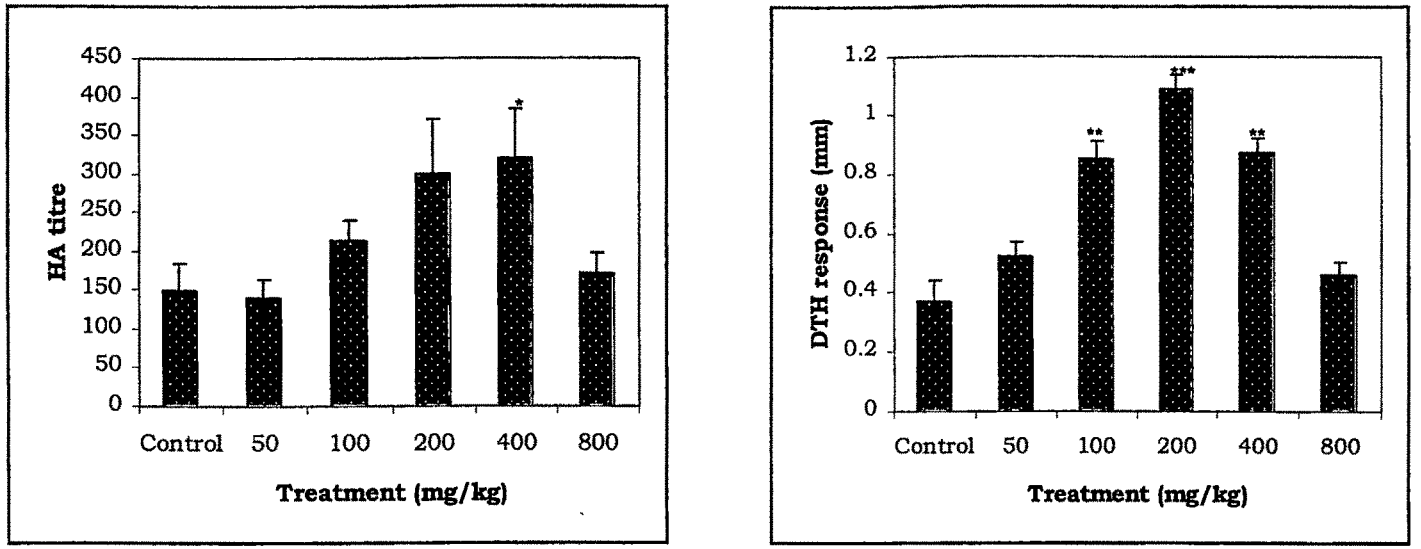


Fig 3.33: Effect of methanol extract of *C. orchioides* on humoral (HA Titre) and cellular immunity (DTH response) using SRBCs as antigen- 15 days pretreatment.

Values are expressed as mean ± SEM.

Group I was compared with groups II, III, IV, V and VI.

*p<0.05; **p<0.01; ***p<0.001.

Table 3.17: Effect of bioactive fraction of *S.indicus* on HA titre and DTH response using SRBCs as antigen-15 days pretreatment.

Groups	Treatment	Dose (mg/kg)	HA titre Mean±S.E.M.	DTH response(mm) Mean paw edema ±S.E.M.
I	Control	-	64.00 ± 014.31	0.313 ± 0.04
II	Bioactive fraction	50	298.67 ± 71.395 $^{N S}$	0.370 ± 0.027 $^{N.S.}$
III	Bioactive fraction	100	384.00 ± 57.243 $^{N S}$	0.388 ± 0.042 $^{N.S.}$
IV	Bioactive fraction	200	640.00 ± 128.00**	0.520 ± 0.089**
V	Bioactive fraction	300	853.33 ± 107.94***	0.456 ± 0.068 $^{N.S.}$
VI	Bioactive fraction	800	448.00 ± 131.16 $^{N S}$	0.337 ± 0.052 $^{N.S.}$
F value			8.362	6.212
P value			0.0001	0.0005

n = 6 mice per group,

Values are expressed as mean ± SEM.

Groups I was compared with groups II, III, IV, V and VI.

$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{N.S.}$ = Non Significant

Table 3.18: Effect of methanol extract of *C. pareira* on HA titre and DTH response using SRBCs as antigen-15 days pretreatment.

Group	Treatment	Dose (mg/kg)	HA titre Mean±S.E.M.	DTH response (mm) Mean paw edema ±S.E.M.
I	Control	-	149.33 ± 35.69	0.37 ± 0.07
II	MeOH Ext.	50	96.00 ± 14.31	0.46 ± 0.08 N.S.
III	MeOH Ext.	100	128.00 ± 28.62	0.76 ± 0.08 N.S.
IV	MeOH Ext.	200	160.00 ± 32.00	0.99 ± 0.06**
V	MeOH Ext.	400	181.33 ± 34.73	1.32 ± 0.17***
VI	MeOH Ext.	800	160.00 ± 32.00	0.91 ± 0.09**
F value			0.9672	12.412
P value			<0.4535 (N.S.)	<0.0001

n = 6 mice per group,

Values are expressed as mean ± SEM.

Groups I was compared with groups II, III, IV, V and VI.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

Table 3.19: Effect of methanol extract of *C. orchioides* on HA titre and DTH response using SRBCs as antigen- 15 days pretreatment.

Group	Treatment	Dose (mg/kg)	HA titre Mean±S.E.M.	DTH response (mm) Mean paw edema ±S.E.M.
I	Control	-	149.33 ± 35.69	0.37 ± 0.07
II	MeOH Ext.	50	138.67 ± 25.69 N.S.	0.52 ± 0.04 N.S.
III	MeOH Ext.	100	213.33 ± 26.98 N.S.	0.85 ± 0.05***
IV	MeOH Ext.	200	298.67 ± 71.39 N.S.	1.09 ± 0.06***
V	MeOH Ext.	400	320.00 ± 64.00*	0.87 ± 0.05***
VI	MeOH Ext.	800	170.67 ± 26.98 N.S.	0.46 ± 0.05 N.S.
F value			2.78	24.078
P value			<0.0309	<0.0001

n = 6 mice per group,

Values are expressed as mean ± SEM.

Groups I was compared with groups II, III, IV, V and VI.

*p<0.05; **p<0.01; ***p<0.001; N.S. = Non Significant

3.4 *IN VITRO* ANTIOXIDANT STUDIES:

Extracts and /or fractions of selected plant *S. indicus, C. pareira and C. orchioides* exhibited different levels of antioxidant activity in the models studied. Results are expressed as % inhibition (Mean ± SEM) of three readings.

3.4.1 DPPH Assay

Methanol extract and its different fractions of *S. indicus* were tested for this assay. Results obtained showed petroleum ether, benzene and chloroform fractions of methanol extract were ineffective in scavenging stable free radical DPPH. Methanol extract and residual methanol fraction of *S. indicus* found effective scavengers of DPPH. The % inhibitions at the concentrations of 250, 500, 750 and 1000 µg/ml of the methanolic extract of the drug were 17.81%, 27.68 %, 34.07 % and 71.70 % respectively. IC_{50} value of methanol extract was found 806.00 µg/ml (Fig 3.35 A). Residual methanol fraction was found more effective than methanol extract with 25.67%, 42.18%, 54.21% and 72.77% inhibition at concentration 100, 200, 300 and 400 µg/ml respectively (Fig 3.35 B). IC_{50} value was found 258.00 µg/ml. Standard antioxidant used was Curcumin, it showed IC_{50} value 52.00 µg/ml (Fig 3.34). Residual methanol fraction was found almost three times more potent than methanol extract and five times less than Curcumin. Petroleum ether fraction gave percentage inhibitions 02.36 %, 05.40 %, 10.85 % and 18.39 % at concentrations 250, 500, 750 and 1000 µg/ml respectively. Benzene fraction gave percentage inhibitions 5.00 %, 5.65 %, 11.20 % and 11.98 % at concentrations 250, 500, 750 and 1000 µg/ml respectively. Chloroform fraction gave percentage inhibitions 1.25 %, 3.85 %, 8.90 % and 10.89 % at concentrations 250, 500, 750 and 1000 µg/ml respectively.

Methanol extract and alkaloidal fraction of roots of *C.pareira* were tested for this assay. Methanol extract and alkaloidal fraction both were found effective in scavenging DPPH in concentration dependent manner. Methanol extract showed 36.52%, 45.04%, 68.21%, 85.00% and 94.11%

inhibition at concentration 100, 200, 400, 600 and 800 µg/ml respectively (Fig 3.36 A). Alkaloidal fraction showed 24.35%, 36.34%, 53.98%, 59.70% and 66.39% at concentrations 20, 40, 60, 80 and 100 µg/ml respectively (Fig 3.36 B). Free radical activity of alkaloidal fraction (IC_{50} value 63.44 µg/ml) was comparable to standard Curcumin (IC_{50} value 52.00 µg/ml), whereas methanol extract (IC_{50} value 235.00 µg/ml) was almost five times less potent than standard.

Methanol extract of *C. orchioides* was subjected for this assay. It showed 4.5%, 15.61%, 41.96%, 67.56% and 83.78% inhibition at concentration 25, 50, 75, 100 and 200 µg/ml respectively (Fig 3.37). IC_{50} value of it was 106.00 µg/ml which is two times more than standard Curcumin.

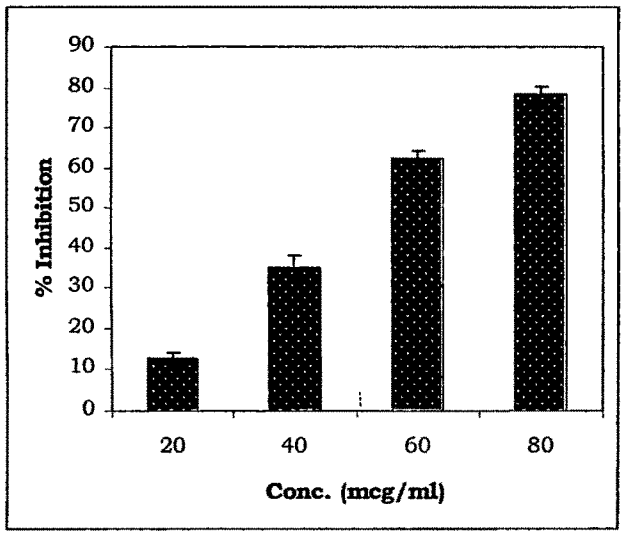
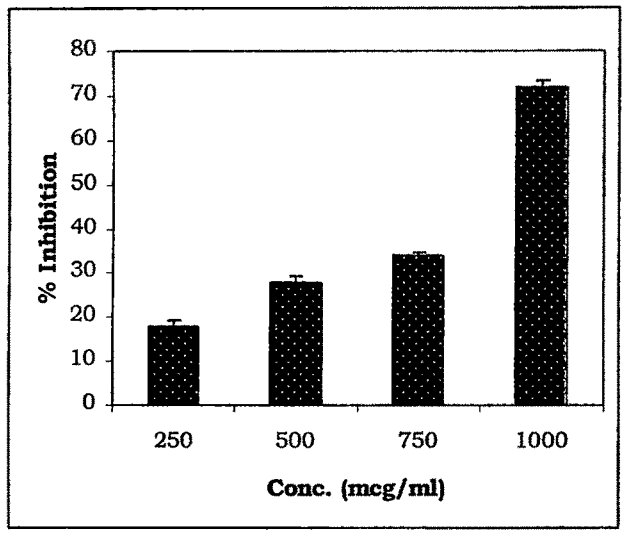
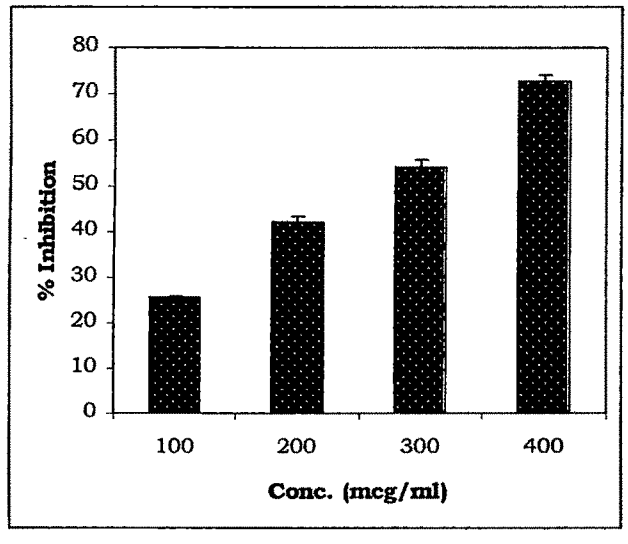


Fig 3.34: Antiradical activity of Curcumin (Standard) observed with DPPH. (n= 3)

IC50of Curcumin was found 52.71 µg/ml.



A



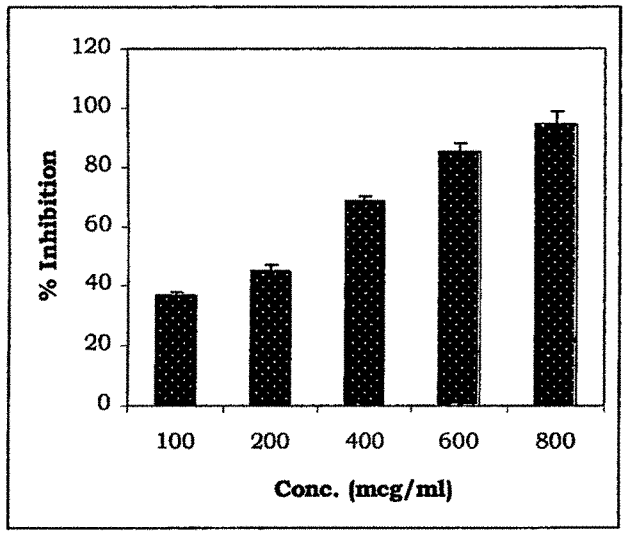
B

Fig 3.35: Antiradical activity of methanol extract (A) and bioactive fraction (B) of *S. indicus* observed with DPPH (n = 3).

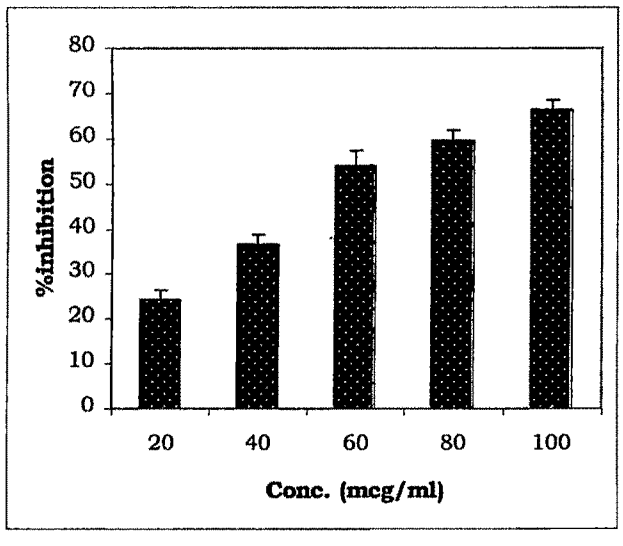
IC_{50} of methanol extract- 806.00 µg/ml

IC_{50} of bioactive fraction- 258.00 µg/ml

IC_{50} of Curcumin- 52.71 µg/ml.



A



B

Fig 3.36: Antiradical activity of methanol extract (A) and alkaloidal fraction (B) of *C. pareira* observed with DPPH (n = 3).

IC_{50} of methanol extract- 235.00 µg/ml

IC_{50} of alkaloidal fraction- 63.44 µg/ml

IC_{50} of Curcumin- 52.71 µg/ml.

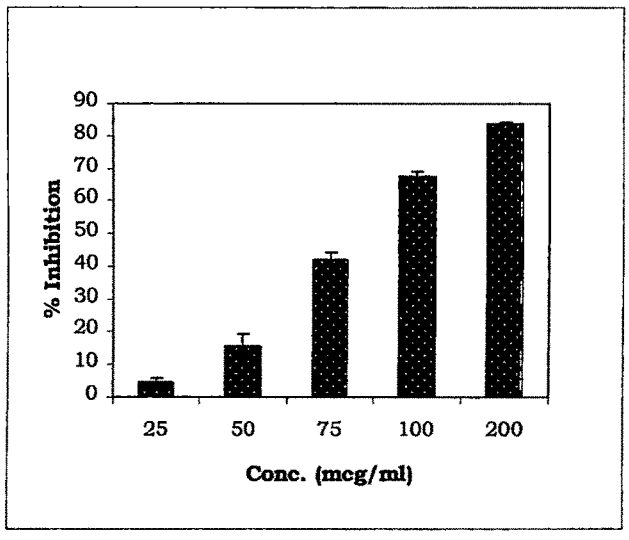


Fig 3.37: Antiradical activity of methanol extract of *C. orchioides* observed with DPPH (n = 3).

IC_{50} of methanol extract: 106.00 µg/ml

IC_{50} of Curcumin: 52.71 µg/ml.

3.4.2 Assay of superoxide scavenging activity

Methanol extract and its different fractions of *S. indicus* were tested for this assay. Results obtained showed petroleum ether, benzene and chloroform fractions of methanol extract were ineffective in scavenging superoxide radical. Petroleum ether fraction showed percentage inhibitions of 2.32 %, 5.50 %, 7.53 % and 8.88 % at concentrations 250, 500, 700 and 1000 µg/ml respectively. Benzene fraction when tested at concentrations 250, 500, 700 and 1000 µg/ml, showed 5.52 %, 5.65 %, 8.85 % and 10.00 % inhibitions respectively. Chloroform fraction gave percentage inhibitions of 4.25 %, 5.56 %, 7.25 % and 12.65 % when tested at concentrations 250, 500, 700 and 1000 µg/ml respectively.

Methanol extract and residual methanol fraction of *S. indicus* found effective scavengers of superoxide. The % inhibitions at the concentrations of 250, 500, 705 and 1000 µg/ml of the methanolic extract of the drug were 27.76%, 39.45%, 49.96 % and 69.30 % respectively (Fig 3.39 A). IC_{50} value of methanol extract was found 687.00 µg/ml. Residual methanol fraction was found more effective than methanol extract with 30.14%, 51.20%, 62.73% and 76.51% inhibition at concentration 50, 100, 150 and 200 µg/ml respectively (Fig 3.39 B). IC_{50} value was found 107.00 µg/ml. Standard antioxidant used was ascorbic acid, it showed IC_{50} value 23.52 µg/ml (Fig 3.38). Residual methanol fraction was found almost six times more potent than methanol extract and four times less than ascorbic acid.

Methanol extract and alkaloidal fraction of roots of *C.pareira* were tested for this assay. Both the tested samples showed concentration dependent effect in scavenging superoxide radical. Alkaloidal fraction (IC_{50} value 31.99 µg/ml) was found more effective in scavenging than methanol extract (IC_{50} value 164.00 µg/ml). Activity of both was found moderate in comparison to standard ascorbic acid (IC_{50} value 23.52µg/ml). Methanol extract showed 24.14%, 35.67%, 44.58%, 59.47% and 69.25% inhibition at concentrations 50, 100, 150, 200 and 250 µg/ml respectively (Fig 3.40 A). Alkaloidal fraction showed 14.54%, 35.62%, 48.69%, 62.35% and 74.28%

inhibition at concentrations 10, 20, 30, 40 and 50 µg/ml respectively (Fig 3.40 B).

Methanol extract of *C. orchioides* was subjected for this assay. It was tested at concentrations 10, 20, 30, 40 and 50 µg/ml with percentage inhibition 23.13%, 47.93%, 50.61%, 60.82% and 71.43% respectively (Fig 3.41). IC_{50} value of extract was found 29.28 µg/ml. Superoxide scavenging activity of *C. orchioides* was comparable to that of standard ascorbic acid.

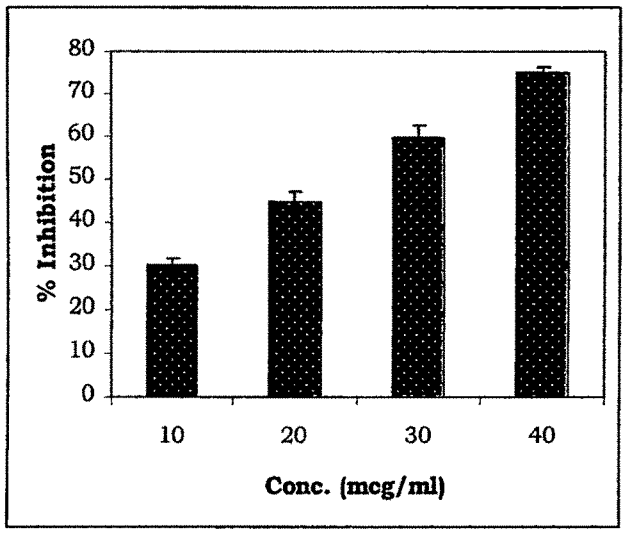
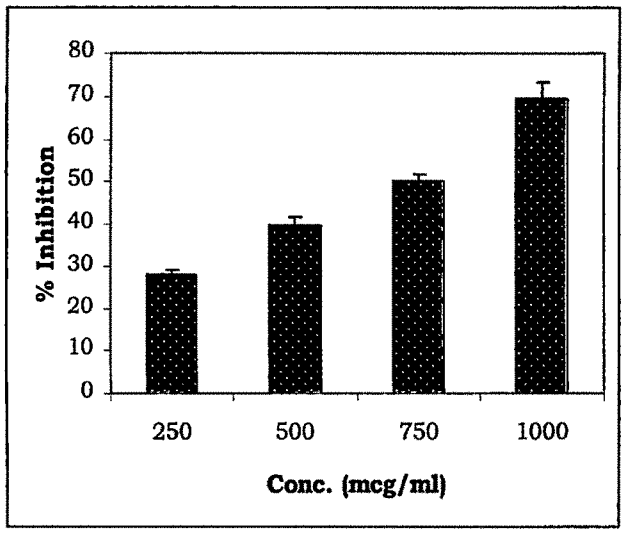
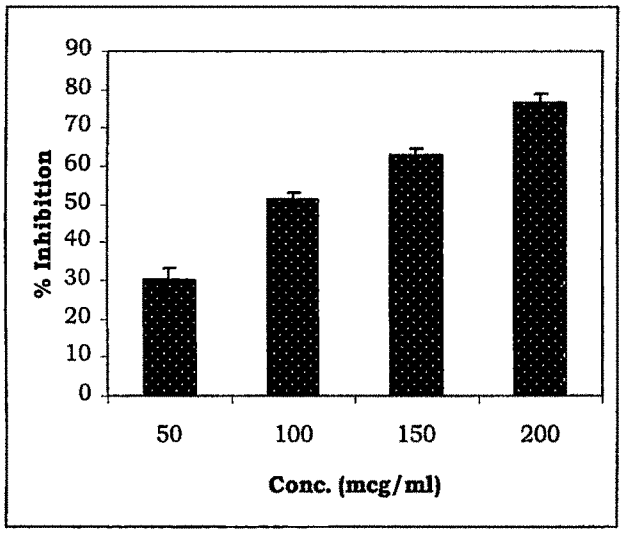


Fig 3.38: Superoxide anion scavenging activity of Ascorbic acid (Standard). (n= 3)

IC_{50} of ascorbic acid was found 23.31 µg/ml.



A



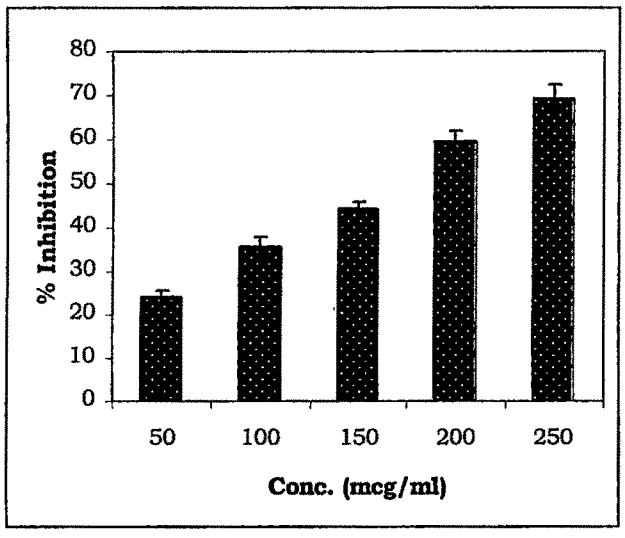
B

Fig 3.39: Superoxide anion scavenging activity of methanol extract (A) and bioactive fraction (B) of *S. indicus* observed with Riboflavin-Light-NBT system (n = 3).

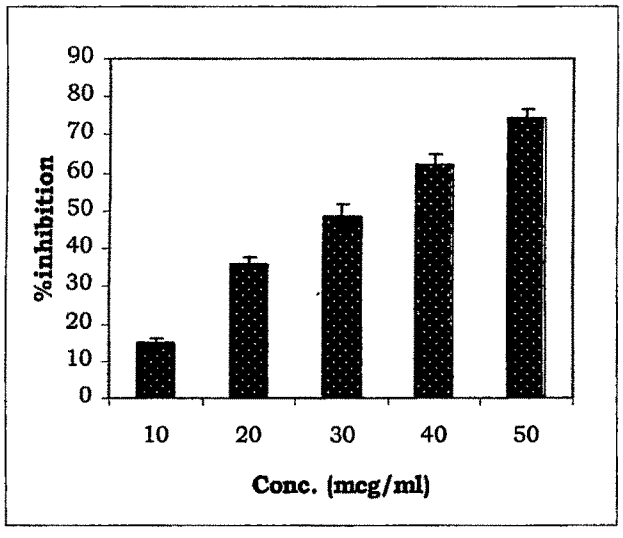
IC_{50} of methanol extract: 687.00 µg/ml

IC_{50} of bioactive fraction: 107.00 µg/ml

IC_{50} of Ascorbic acid: 23.52 µg/ml.



A



B

Fig 3.40: Superoxide anion scavenging activity of methanol extract (A) and alkaloidal fraction (B) of *C. pareira* observed with Riboflavin-Light-NBT system (n = 3).

IC_{50} of methanol extract: 164.00 µg/ml

IC_{50} of alkaloidal fraction: 31.99 µg/ml

IC_{50} of Ascorbic acid: 23.52 µg/ml.

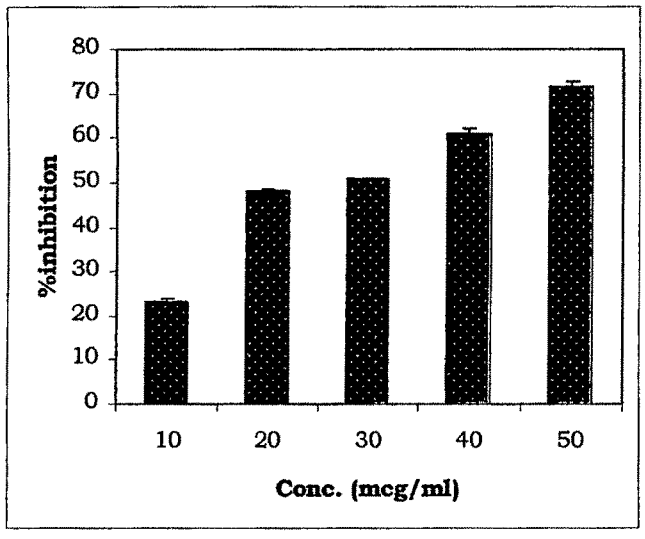


Fig 3.41: Superoxide anion scavenging activity of methanol extract of *C. orchioides* observed with Riboflavin-Light-NBT system (n = 3).

IC_{50} of methanol extract: 29.28 µg/ml

IC_{50} of Ascorbic acid: 23.52 µg/ml.

3.4.3 Assay of nitric oxide scavenging activity

Methanol extract and its different fractions of *S. indicus* were tested for this assay. Methanol extract and none of its fraction found effective in scavenging nitric oxide radical. All extracts and fractions were tested at concentrations 250, 500, 700 and 1000 µg/ml. Methanol extract gave percentage inhibitions of 5.00 %, 8.58 %, 15.54 % and 22.50 %, petroleum ether fraction gave 1.05 %, 2.21 %, 2.25 % and 3.00 %, benzene fraction gave 2.22 %, 3.45 %, 4.52 % and 5.00 %, chloroform fraction gave 1.00 %, 4.00 %, 3.50 % and 4.56 %, whereas methanol fraction gave 10.25 %, 11.24 %, 15.87 % and 20.00 % at respective tested concentrations i.e. 250, 500, 700 and 1000 µg/ml.

Methanol extract and alkaloidal fraction of roots of *C.pareira* were tested for this assay. Both of the tested samples could not able to scavenge nitric oxide radical at tested concentrations. Percentage inhibitions obtained with methanol extract were 0.98 %, 2.50 %, 3.25 % and 5.64 %, whereas with alkaloidal fraction 1.25 %, 1.98 %, 2.30 % and 5.85 % at concentrations 250, 500, 750 and 1000 µg/ml respectively.

Methanol extract of *C. orchioides* was subjected for this assay. It was found effective scavenger of nitric oxide with 43.28%, 47.40%, 50.69%, 56.68% and 65.82% inhibition at concentrations 60, 80, 100, 120 and 140 µg/ml respectively (Fig 3.43). IC_{50} value was found 90.96 µg/ml. It had four times less activity than standard used. Curcumin was used as standard and had IC_{50} value 21.59 µg/ml (Fig 3.42).

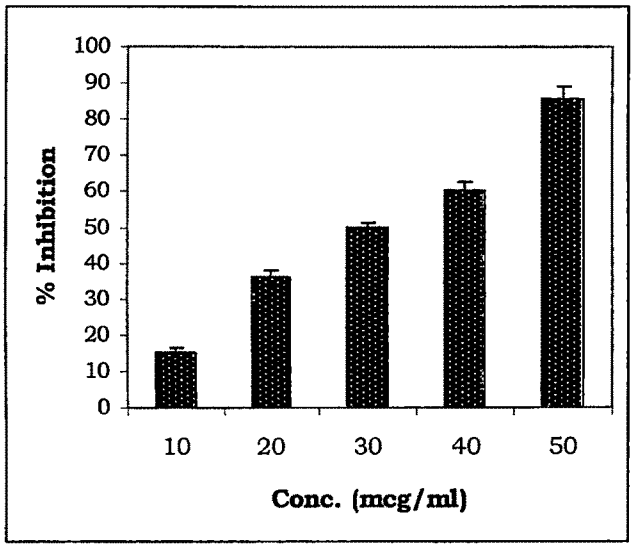


Fig 3.42: Nitric oxide scavenging activity of standard antioxidant Curcumin.

(n= 3)

IC_{50} of Curcumin was found 21.59 µg/ml.

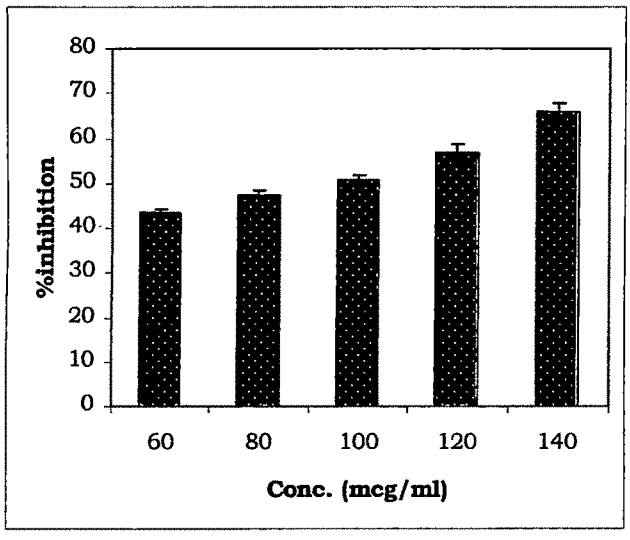


Fig 3.43: Nitric oxide scavenging activity of methanol extract of *C. orchioides.* (n= 3)

IC_{50} of methanol extract: 90.96 µg/ml

IC_{50} of Curcumin: 21.59 µg/ml.

3.4.4 Reducing power determinations

Methanol extract and its different fractions of *S. indicus* were tested for reducing power determinations. Methanol extract increased absorbance of the test medium in dose dependent manner viz., 0.100, 0.220 and 0.313 at concentrations 5.0, 10 and 15 mg/ml respectively, whereas methanol fraction gave absorbance of 0.120, 0.250 and 0.353 at concentrations 5.00, 10 and 15 mg/ml respectively. All the remaining fractions failed to show any reducing power. Standard used for this assay was ascorbic acid, showed absorbance of 0.430 at concentration 0.3 mg/kg.

Reducing power determinations carried out on methanol extract and alkaloidal fraction of *C. pareira* showed increased absorbance with increasing concentration. Methanol extract showed absorbance of 0.230, 0.450 and 0.500 at concentration 5, 10 and 15 mg/ml respectively, whereas alkaloidal fraction showed absorbance of 0.200, 0.401, and 0.558 at the same concentration as that of methanol extract respectively. Activity of both the tested extracts was comparable with activity of standard.

Methanol extract of *C. orchioides* showed increased absorbance with increasing concentrations when subjected to reducing power determinations. It showed absorbance of 0.105, 0.258 and 0.363 at concentrations 5, 10 and 15 mg/ml respectively. Activity was less compared to standard.

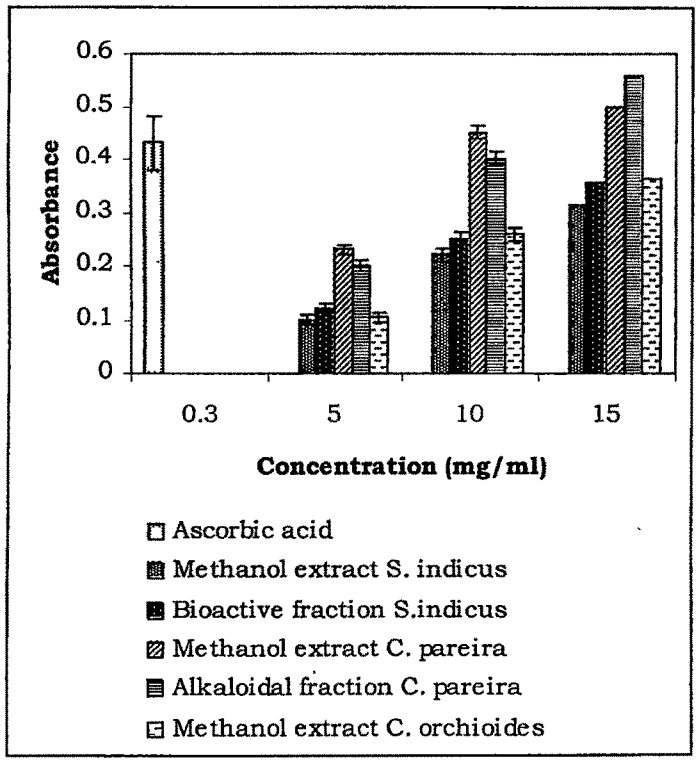


Fig 3.44: Reducing power determinations of extracts of *S. indicus*, *C. pareira* and *C. orchioides*. (n=3)

Ascorbic acid (0.3 mg/ml) was used as a standard.

3.4.5 Measurement of inhibition of lipid peroxidation induced by Iron/ADP/Ascorbate complex in rat liver homogenate

Methanol extract and its different fractions of *S. indicus* were tested for measuring inhibition of lipid peroxidation induced by Iron/ADP/Ascorbate complex in rat liver homogenate. Petroleum ether, benzene and chloroform fractions were failed to offer any protection against lipid peroxidation. Petroleum ether fraction gave percentage inhibitions of 2.50 %, 5.24 %, 10.65 % and 20.24 %, benzene fraction gave 1.25 %, 2.98 %, 3.63 % and 5.00 %, whereas chloroform fraction gave 0.99 %, 2.85 %, 8.53 % and 10.00 % inhibitions at concentrations 250, 500, 750 and 1000 µg/ml respectively.

Methanol extract and residual methanol fraction showed dose dependent inhibitory response against lipid peroxidation. Methanol extract showed 18.65%, 39.25%, 65.00%, 75.58% and 80.25% inhibition at concentration 200, 400, 600, 800 and 1000 µg/ml respectively with IC_{50} value of 528.00 µg/ml (Fig 3.46 A). Residual methanol fraction showed 40.37%, 52.15%, 66.23% and 73.25% inhibition at concentration 200, 300, 400 and 500 µg/ml respectively with IC_{50} value 290 µg/ml (Fig 3.46 B). IC_{50} value of standard ascorbic acid was found 30.00 µg/ml (Fig 3.45). Tested extracts showed moderate to mild activity when compared to standard.

Methanol extract and alkaloidal fraction of roots of *C.pareira* were tested for this assay. Methanol extract showed IC_{50} value 91.00 µg/ml, whereas alkaloidal fraction showed IC_{50} value 62.00 µg/ml. Methanol extract showed 12.32%, 23.51%, 36.64%, 59.27% and 68.24% inhibition of lipid peroxidation at concentrations 25, 50, 75, 100 and 125 µg/ml respectively (Fig 3.47 A). Alkaloidal fraction showed 25.38%, 45.67%, 60.24%, 75.02% and 80.29% inhibition at concentrations 25, 50, 75, 100 and 125 mg/ml respectively (Fig 3.47 B). Activity of methanol extract was three times and alkaloidal fraction was two times less than standard ascorbic acid.

Methanol extract of *C. orchioides* was subjected for this assay. Lipid peroxidation induced by Iron/ADP/ascorbate complex was effectively inhibited by methanol extract of *C. orchioides*. It showed 14.24%, 24.33%, 39.93%, 51.14% and 67.57% inhibition at concentrations 25, 50, 75, 100 and 125 µg/ml respectively. IC_{50} value was found 94.78 µg/ml (Fig 3.48).

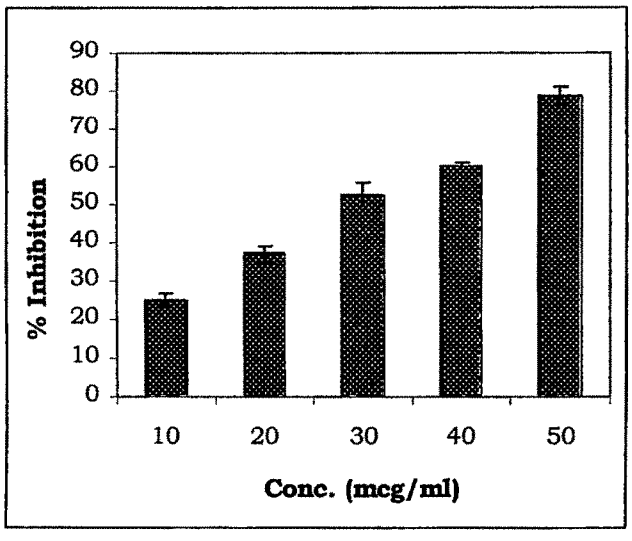
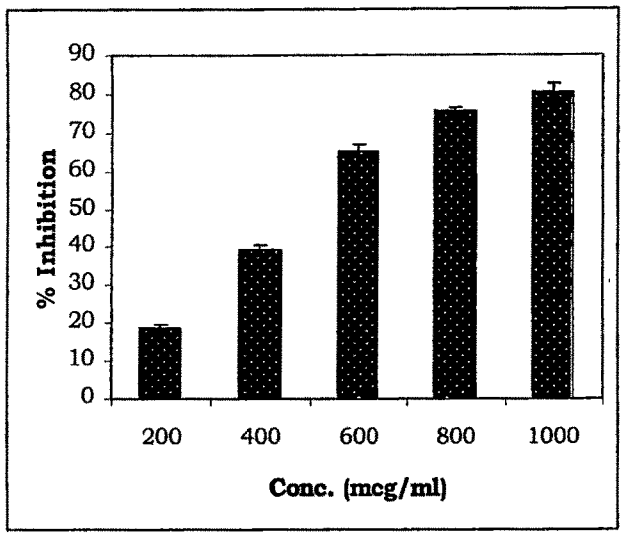


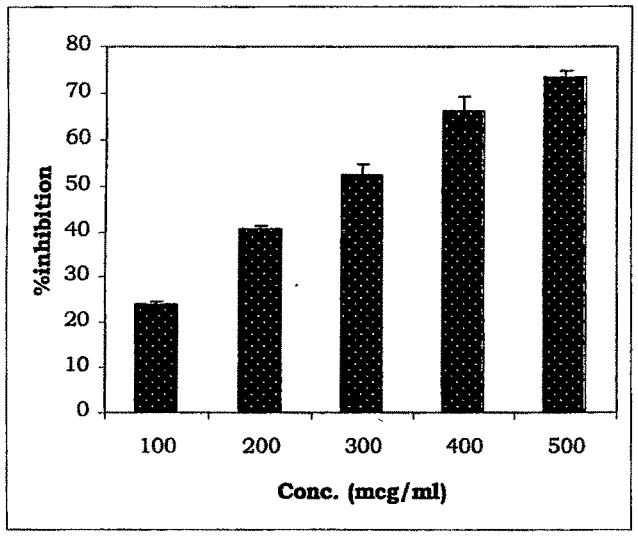
Fig 3.45: Measurement of Inhibition of lipid peroxidation induced by iron/ADP/Ascorbate system in rat liver homogenate by standard antioxidant, ascorbic acid.

(n= 3)

IC_{50} of Ascorbic acid was found 29.51 µg/ml.



A



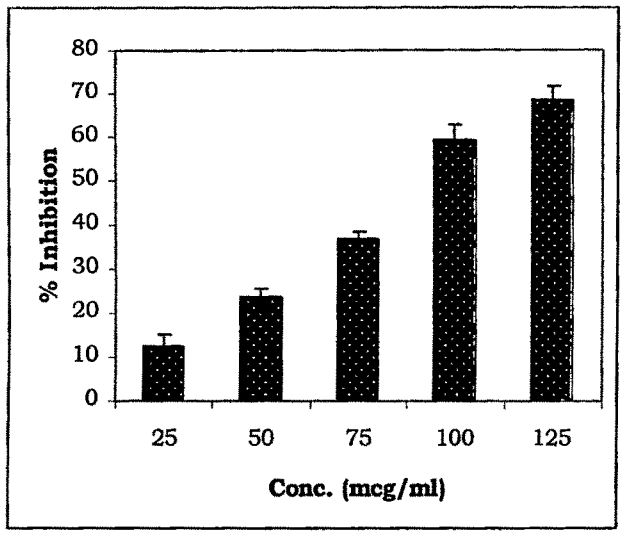
B

Fig 3.46: Measurement of Inhibition of lipid peroxidation induced by iron/ADP/Ascorbate system in rat liver homogenate by methanol extract (A) and bioactive fraction (B) of *S. indicus*. (n= 3).

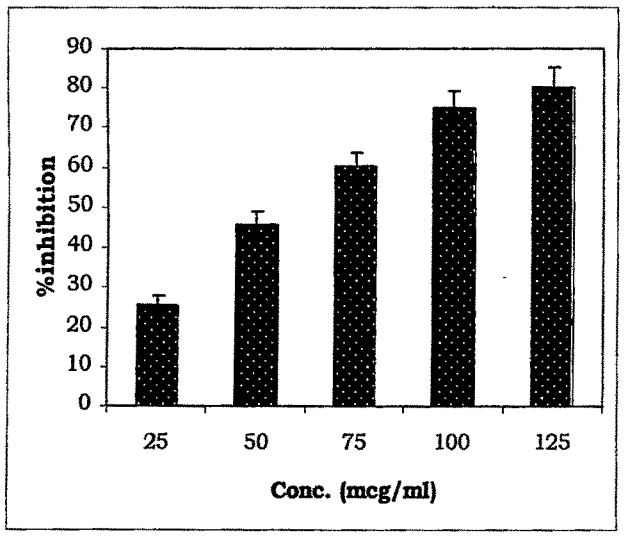
IC_{50} of methanol extract: 528.00 µg/ml

IC_{50} of bioactive fraction: 290 µg/ml

IC_{50} of Ascorbic acid: 30.00 µg/ml



A



B

Fig 3.47: Measurement of Inhibition of lipid peroxidation induced by iron/ADP/Ascorbate system in rat liver homogenate by methanol extract (A) and alkaloidal fraction (B) of *C. pareira.* (n= 3)

IC_{50} of methanol extract: 91.00 µg/ml

IC_{50} of alkaloidal fraction: 62.00 µg/ml

IC_{50} of Ascorbic acid: 30.00 µg/ml

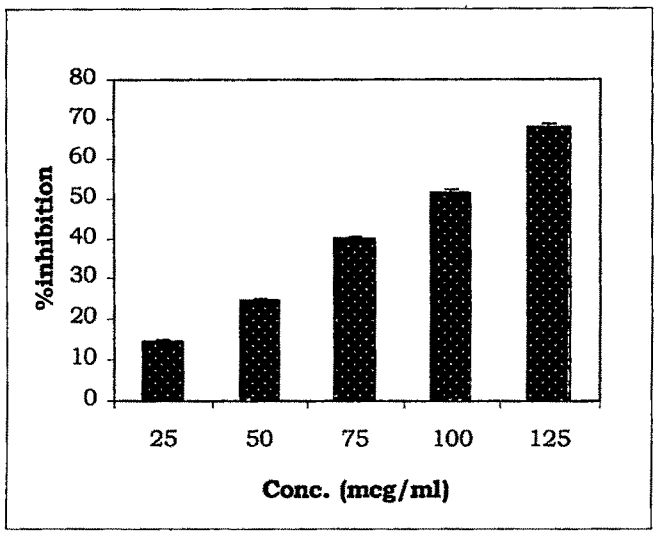


Fig 3.48: Measurement of Inhibition of lipid peroxidation induced by iron/ADP/Ascorbate system in rat liver homogenate by methanol extract of *C. orchioides*. (n= 3)

IC_{50} of methanol extract: 94.78 µg/ml

IC_{50} of Ascorbic acid: 30.00 µg/ml

3.5 HPTLC Fingerprinting profile of the extracts and / or fractions

3.5.1 HPTLC profile of methanol extract of *S. indicus*

Solvent system 1: Plate spotted with methanol extract of S. indicus was first run into solvent system, toluene: ethyl acetate (7:3). Scanning of plate at 254 nm revealed presence of two spots with R_f 0.09 and 0.67. These compounds had λ_{max} 325 and 700 respectively. Respective percentages of these compounds were 98.80 and 1.20.

Scanning at 366 nm of this plate revealed presence of 11 spots. R_f of these compounds were 0.04, 0.10, 0.22, 0.29, 0.36, 0.45, 0.56, 0.67, 0.80, 0.89 and 0.99 with λ_{max} at 315, 328, 330, 329, 699, 699, 699, 700, 699, 325 and 329 nm respectively. Compounds located at R_f 0.22, 0.36, 0.45, 0.56 and 0.67 were having substantiate proportion only i.e. 11.91, 13.25, 17.30, 15.30 and 13.34 % respectively (Table 3.21, Fig 3.49).

Solvent system 2: Second solvent system used to resolute compounds from methanol extract was ethyl acetate: methanol: water (100: 13.5: 10). Densitometric scanning at 254 nm revealed presence of three compounds. R_f of these compounds were 0.03, 0.08 and 0.28 with maximal absorbance at wavelength 309, 331 and 329 nm respectively. Compounds having R_f 0.03 and 0.28 were major compound with 42.15 and 50.09 % respectively.

Scanning at 366 nm with fluorescence mode reveled presence of 14 compounds with R_f 0.02, 0.6, 0.09, 0.14, 0.21, 0.27, 0.33, 0.42, 0.52, 0.60, 0.73, 0.81, 0.89 and 0.95. Respective wavelength maxima of these compounds were at 322, 329, 328, 328, 330, 329, 346, 414, 700, 698, 698, 200, 200 and 200 nm. Compounds with major percentages were 0.42 (14.56 %), 0.52 (13.55 %), 0.60 (12.07 %) and 0.95 (10.46 %)(Table 3.21, Fig 3.49).

Solvent system 3: Third solvent system used was more polar to resolute all polar compounds, n-butanol: glacial acetic acid (6: 2: 2). Scanning at 254 nm showed eight compounds. R_f of these compounds were 0.03, 0.26, 0.34, 0.52, 0.58, 0.71, 0.82 and 0.94 with maximal absorbance at wavelength

693, 699, 700, 200, 200, 330, 329 and 264 nm respectively. Compounds with major percentages were 0.71 (19.06 %), 0.82 (21.72 %) and 0.94 (41.64 %).

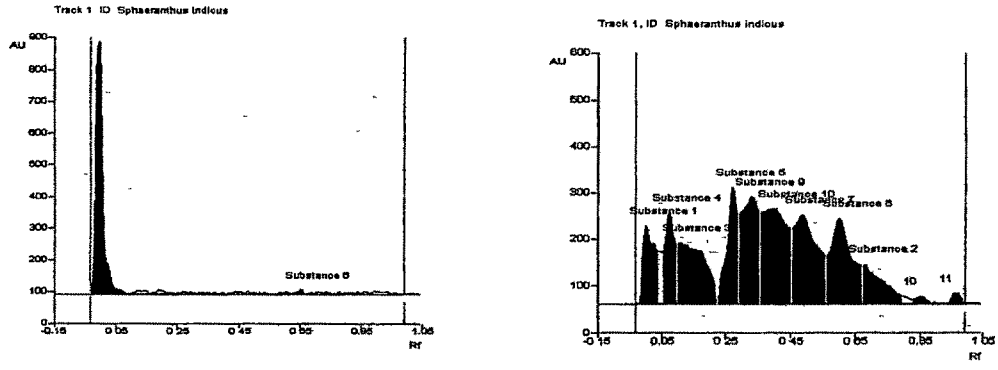
Scanning at 366 nm revealed the presence of eight compounds. R_f of these compounds were 0.14, 0.30, 0.54, 0.57, 0.65, 0.78, 0.86 and 0.94 with maximal absorbance at wavelength 700, 699, 200, 200, 200, 200, 200 and 200 nm respectively. Compounds with major percentages were 0.54 (28.51 %), 0.65 (14.29 %), 0.78 (11.13 %), 0.86 (11.06 %) and 0.94 (12.21 %)(Table 3.21, Fig 3.49).

Table 3.20: HPTLC finger print profile of methanol extract of *S. indicus*.

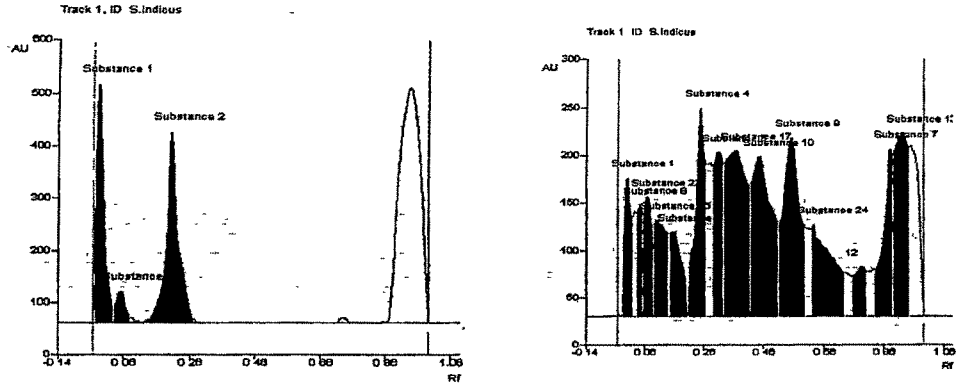
Scanned at	Solvent system 1			Solvent system 2			Solvent system 3		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
	0.09	325	98.80	0.03	309	42.15	0.03	693	1.72
	0.67	700	1.20	0.08	331	7.16	0.26	699	3.08
				0.28	329	50.69	0.34	700	6.88
254 nm							0.52	200	4.37
							0.58	200	1.52
							0.71	330	19.06
							0.82	329	21.72
							0.94	264	41.64
	0.04	315	6.70	0.02	322	3.65	0.14	700	4.85
	0.10	328	6.55	0.06	329	2.66	0.30	699	8.83
	0.22	330	11.91	0.09	328	4.03	0.54	200	28.51
	0.29	329	8.87	0.14	328	5.17	0.57	200	9.13
366 nm	0.36	699	13.25	0.21	330	3.83	0.65	200	14.29
	0.45	699	17.30	0.27	329	7.92	0.78	200	11.13
	0.56	699	15.30	0.33	346	6.26	0.86	200	11.06
	0.67	700	13.34	0.42	414	14.56	0.94	200	12.21
	0.80	699	5.56	0.52	700	13.55			
	0.89	325	0.52	0.60	698	12.07			
	0.99	329	0.66	0.73	698	7.54			
				0.81	200	2.26			
				0.89	200	6.05			
				0.95	200	10.46			

Solvent system 1: Toluene: ethyl acetate (7:3)
Solvent system 2: Ethyl acetate: methanol: water (100:13.5:10)
Solvent system 3: n-butanol: glacial acetic acid (6:2:2)

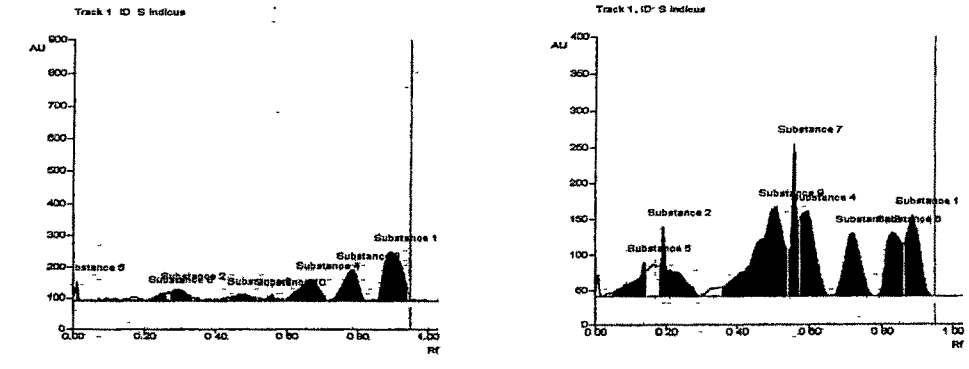
Wavelength 254 **Wavelength 366**



Solvent system 1



Solvent system 2



Solvent system 3

Fig 3.49: Chromatograms of methanol extract of *S. indicus*.

3.5.2 HPTLC fingerprint profile of petroleum ether fraction of *S. indicus*

Solvent system 1: Petroleum ether fraction resoluted in solvent system; hexane: ethyl acetate (9:1). Scanning at wavelength 254 nm revealed four clearly separated spots. R_f of the separated compounds were 0.03, 0.13, 0.58 and 0.97 with maximal absorbance at wavelength 290, 264, 691 and 224 nm and % 2.39, 43.19, 38.00 and 16.42 respectively.

Scanning of plate at wavelength 366 showed presence of five components. R_f of these compounds were 0.03, 0.18, 0.30, 0.38 and 0.44 with maximal absorbance at wavelength 611, 521, 597, 606 and 641 nm and % of these were 14.70, 13.79, 30.29, 16.37 and 24.85 respectively (Table 3.21, Fig 3.50).

Solvent system 2: Another solvent system used to resolute remaining components from petroleum ether fraction was; toluene: ethyl acetate (7:3). Plate when subjected to scan at 254 nm revealed the presence of five compounds. R_f of these compounds were 0.51, 0.57, 0.80, 0.91 and 0.98, with $\lambda_{\square max}$ 268, 275, 263, 223 and 219. Percentage of the components located at R_f 0.51 and 0.91 was major i.e. 18.57 and 63.88 respectively.

Scanning of plate at 366 nm showed seven clearly separated compounds. R_f of these components were 0.18, 0.25, 0.46, 0.64, 0.76, 0.98 and 0.97 with $\lambda_{\square max}$ 266, 617, 270, 200, 270, 270 and 200 respectively. Last four components showed major percentage with 35.86, 13.54, 21.42 and 10.11 respectively (Table 3.21, Fig 3.50).

3.5.3 HPTLC fingerprint profile of benzene fraction of *S. indicus*

Solvent system 1: Solvent system used to resolute the components present in benzene fractions was hexane: ethyl acetate (9:1). Scanning at short wavelength 254 nm showed presence of two compounds with R_f 0.04 and 0.57, having percentage 2.54 and 97.56 and $\lambda_{\square max}$ 213 and 258 respectively.

Scanning at long wavelength, 366 revealed presence of two compounds with R_f 0.10 and 0.28, having percentage 50.10 and 49.90 and λ_{max} 611 and 624 respectively (Table 3.22, Fig 3.51).

Solvent system 2: Second solvent system used to resolute remaining compounds from benzene fraction was toluene: ethyl acetate (7:3). Plate when scanned at wavelength 254 nm, revealed presence of five compounds. R_f of these compounds were 0.07, 0.21, 0.46, 0.91 and 0.97, with λ_{max} 628, 700, 219, 261 and 226. Two compounds located at R_f 0.91 and 0.97 had major percentage calculated according to their area, 63.46 and 19.77 % respectively.

Plate when scanned at wavelength 366 nm showed presence of seven compounds. R_f of these compounds were 0.13, 0.25, 0.37, 0.47, 0.59, 0.75 and 0.88, with λ_{max} 270, 606, 606, 218, 606, 261 and 278. Respective percentages of these compounds were 4.59, 11.01, 17.97, 22.33, 23.04, 6.97 and 14.09 (Table 3.22, Fig 3.51).

3.5.4 HPTLC fingerprint profile of chloroform fraction of *S. indicus*

Solvent system 1: Plate spotted with chloroform fraction was run into hexane: ethyl acetate (9:1). Scanning at 254 nm revealed the presence of four compounds. R_f of these compounds were 0.60, 0.74, 0.84 and 0.94 with λ_{max} 259, 652, 690 and 223. Compound located at R_f 0.60 was major compound with 91.94 %.

Scanning at wavelength 366 nm showed three compounds. R_f of these compounds were 0.10, 0.28 and 0.55 with λ_{max} 518, 606 and 615. Respective percentages of these compounds were 39.59, 32.56 and 27.85 % (Table 3.23, Fig 3.52).

Solvent system 2: Another solvent system used to resolute remaining compounds from chloroform fraction was toluene: ethyl acetate (7:3). Plate when scanned at 254 nm, revealed six spots separated clearly. R_f of these

compounds were 0.10, 0.22, 0.46, 0.65, 0.93 and 0.97 with λ_{max} 275, 253, 219, 249, 261 and 226. Respective percentages of these compounds were 3.67, 17.61, 3.08, 11.55, 54.78 and 9.32 %.

Scanning at 366 nm showed presence of six compounds. R_f of these compounds were 0.13, 0.28, 0.36, 0.46, 0.62 and 0.87 with λ_{max} 610, 606, 620, 612, 613 and 606. Respective percentages of these compounds were 7.70, 28.09, 18.91, 17.69, 19.67 and 6.95 % (Table 3.23, Fig 3.52).

3.5.5 HPTLC fingerprint profile of Methanol fraction of *S. indicus*

Solvent system 1: Methanol fraction of *S. indicus* applied on TLC plate and resoluted in solvent system; toluene: ethyl acetate (7:3) and detected at 254 and 366 wavelength. Plate when scanned at 254 showed presence of seven compounds. R_f of these compounds were 0.1, 0.19, 0.24, 0.36, 0.69, 0.81 and 0.90 with λ_{max} 319, 348, 312, 607, 580, 233 and 332 respectively. Major compound calculated from its area was found having R_f 0.90 with 75.10 % and other at R_f 0.81 having 9.68 %, rest other compounds had percentage less than 6.

Plate when scanned at 366 nm with fluorescence mode it showed resolution of eight compounds. R_f of these compounds were 0.07, 0.11, 0.15, 0.23, 0.48, 0.56, 0.90 with λ_{max} 323, 313, 335, 309, 580, 613, 223 and 332 respectively. Major compounds calculated from their areas were located at R_f 0.23, 0.48, 0.56 and 0.90 with % 23.36, 30.32, 24.37 and 20.15 respectively (Table 3.24, Fig 3.53).

Solvent system 2: Methanol fraction subjected to resolute in solvent system; ethyl acetate: methanol: water (100: 13.5: 10). Plate when scanned at 254 nm using absorbance mode gave three spots at R_f 0.32, 0.45 and 0.75 with λ_{max} 330, 203 and 200 respectively. Percentages of these compounds calculated from areas were found 64.30, 5.31 and 30.39 respectively.

Plate when subjected to scanning at 366 nm using fluorescence mode gave three compounds at R_f 0.09, 0.36 and 0.42 with λ_{max} 328, 329 and 203 respectively. Major compound out of these three was located at R_f 0.36 with 93% (Table 3.24, Fig 3.53).

Solvent system 3: The solvent system used was n-butanol: glacial acetic acid (6:2:2). Scanning plate at 254 nm showed resolution of seven compounds. R_f of these compounds were 0.23, 0.31, 0.44, 0.49, 0.60, 0.81 and 0.91 with λ_{max} 200, 323, 318, 324, 328, 228 and 327 respectively. Compounds having major percentages were at R_f 0.60, 0.81 and 0.91 with % of 12.31, 47.09 and 27.63 respectively. Rest of the compounds had lesser proportions.

Plate when subjected to scanning at 366 nm using fluorescence mode gave ten compounds at R_f 0.11, 0.14, 0.35, 0.38, 0.49, 0.56, 0.72, 0.78, 0.89 and 0.95 with λ_{max} 324, 323, 324, 317, 327, 327, 329, 200, 296 and 228 respectively. Compounds located at R_f 0.56, 0.72 and 0.89 were having major percentage i.e. 11.11, 54.14 and 13.59, respectively (Table 3.24, Fig 3.53).

Table 3.21: HPTLC fingerprint profile of petrol ether fraction of methanol extract of *S. indicus*

Scanned at	Solvent system 1			Solvent system 2		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.03	290	2.39	0.51	268	18.57
	0.13	267	43.19	0.57	275	3.40
	0.58	691	38.00	0.80	263	4.45
	0.97	224	16.42	0.91	223	63.88
				0.98	219	9.91
366 nm	0.04	611	14.70	0.18	266	4.49
	0.18	521	13.79	0.25	617	5.66
	0.30	597	30.29	0.46	270	9.03
	0.38	606	16.37	0.64	200	35.86
	0.44	641	24.85	0.76	270	13.54
				0.89	270	21.42
				0.97	200	10.11

Solvent system 1: Hexane: ethyl acetate (9:1)
Solvent system 2: Toluene: ethyl acetate (7:3)

Table 3.22: HPTLC fingerprint profile of benzene fraction of methanol extract of *S. indicus*

Scanned at	Solvent system 1			Solvent system 2		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.04	213	2.54	0.07	628	1.45
	0.57	258	97.56	0.21	700	7.49
				0.46	219	7.82
				0.91	261	63.46
				0.97	226	19.77
366 nm	0.10	611	50.10	0.13	270	4.59
	0.28	624	49.90	0.25	606	11.01
				0.37	606	17.97
				0.47	218	22.33
				0.59	606	23.04
				0.75	261	6.97
				0.88	278	14.09

Solvent system 1: Hexane: ethyl acetate (9:1)
Solvent system 2: Toluene: ethyl acetate (7:3)

Table 3.23: HPTLC fingerprint profile of chloroform fraction of methanol extract *S. indicus*

Scanned at	Solvent system 1			Solvent system 2		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.60	259	91.94	0.10	275	3.67
	0.74	652	2.00	0.22	253	17.61
	0.84	690	3.12	0.46	219	3.08
	0.94	223	2.94	0.65	249	11.55
				0.93	261	54.78
				0.97	226	9.32
366 nm	0.10	518	39.59	0.13	606	7.70
	0.28	606	32.56	0.28	610	28.09
	0.55	615	27.85	0.36	620	18.91
				0.46	612	17.69
				0.62	613	19.67
				0.87	606	6.95

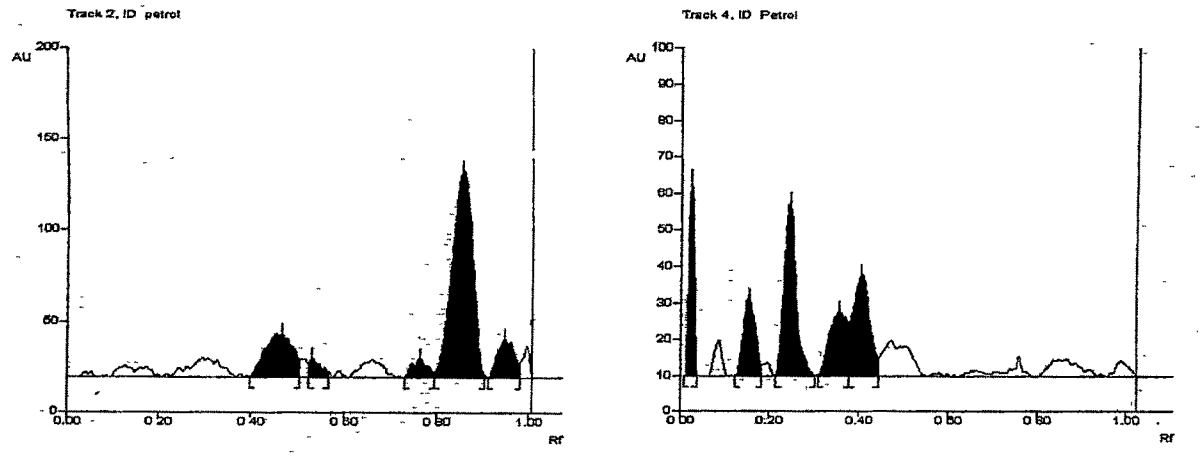
Solvent system 1: Hexane: ethyl acetate (9:1)
Solvent system 2: Toluene: ethyl acetate (7:3)

Table 3.24: TLC finger printing profile of bioactive fraction of *S.indicus*.

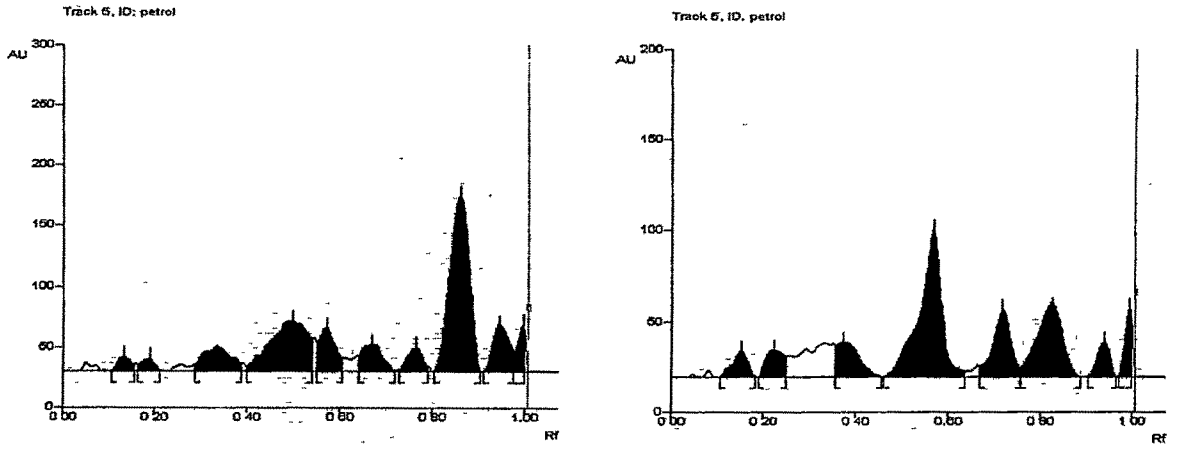
Scanned at	Solvent system 1			Solvent system 2			Solvent system 3		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
	0.10	319	3.68	0.32	330	64.30	0.23	200	0.98
	0.19	348	1.71	0.45	203	5.31	0.31	323	2.60
	0.24	312	5.90	0.75	200	30.39	0.44	318	8.13
254 nm	0.35	607	3.19				0.49	324	1.24
	0.69	580	0.74				0.60	328	12.31
	0.81	233	9.68				0.81	228	47.09
	0.90	332	75.10				0.91	327	27.63
	0.07	323	8.54	0.09	328	4.87	0.11	324	3.60
	0.11	313	5.96	0.36	329	92.34	0.14	323	1.90
	0.15	335	7.45	0.42	203	3.79	0.35	324	6.90
	0.23	309	23.36				0.38	317	1.21
366 nm	0.38	580	30.32				0.49	327	2.17
	0.49	613	24.37				0.56	327	11.11
	0.56	223	7.41				0.72	329	54.14
	0.90	332	20.15				0.78	200	4.35
							0.89	296	13.59
							0.95	228	1.03

Solvent system 1. Toluene/Ethyl acetate (7:3 v/v); Solvent system 2. Ethyl acetate/Methanol/Water (10:1.35:1.00 v/v); Solvent system 3. n-butanol/glacial acetic acid/water (6:2:2 v/v).

Wavelength -254 **Wavelength- 366**



Solvent system 1

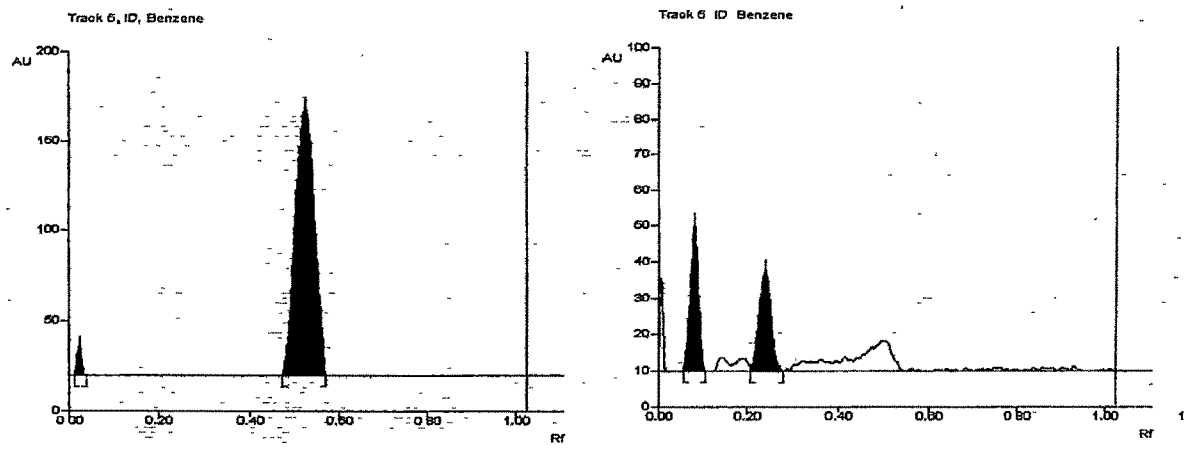


Solvent system 2

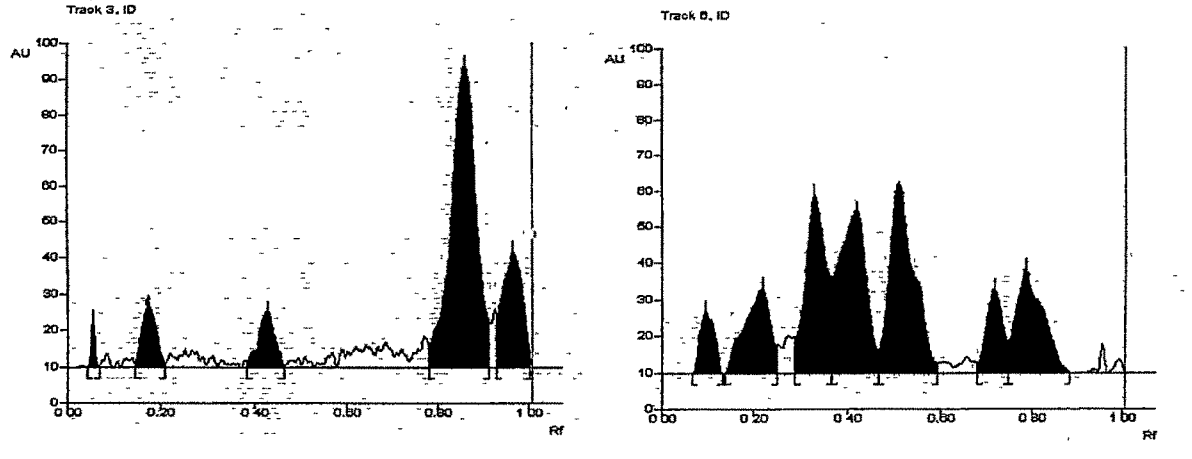
Fig 3.50: Chromatograms of petroleum ether fraction of methanol extract of *S. indicus*.

Solvent system 1: Hexane: ethyl acetate (9:1)
Solvent system 2: Toluene: ethyl acetate (7:3)

Wavelength -254 **Wavelength- 366**



Solvent system 1

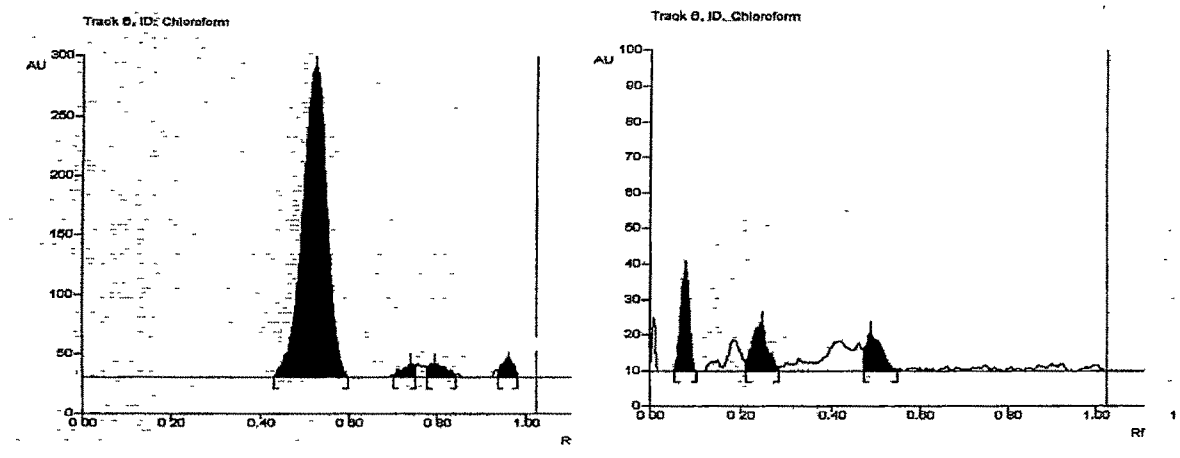


Solvent system 2

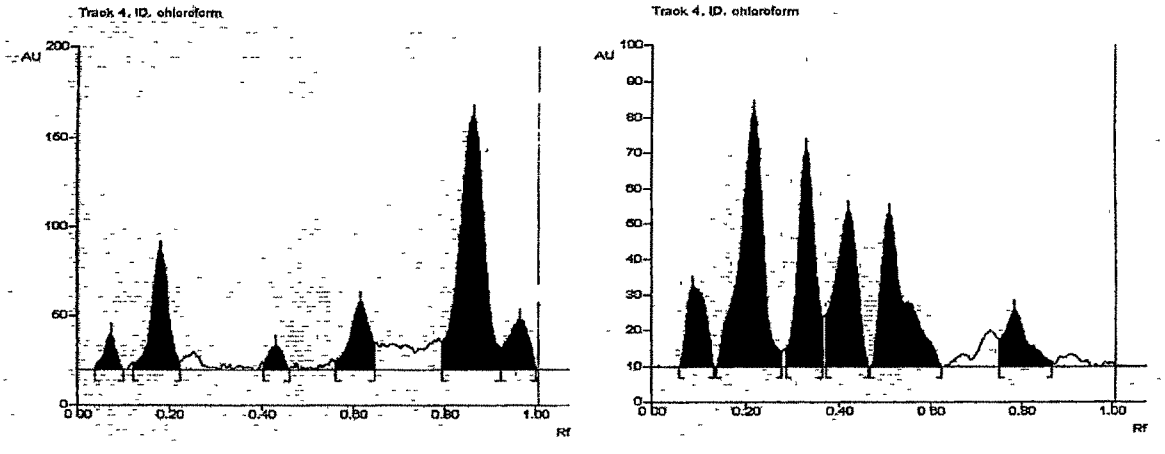
Fig 3.51: Chromatograms of benzene fraction of methanol extract of *S. indicus*.

Solvent system 1: Hexane: ethyl acetate (9:1)
Solvent system 2: Toluene: ethyl acetate (7:3)

Wavelength -254 **Wavelength- 366**



Solvent system 1



Solvent system 2

Fig 3.52: Chromatograms of chloroform fraction of methanol extract of *S. indicus*.

Solvent system 1: Hexane: ethyl acetate (9:1)
Solvent system 2: Toluene: ethyl acetate (7:3)

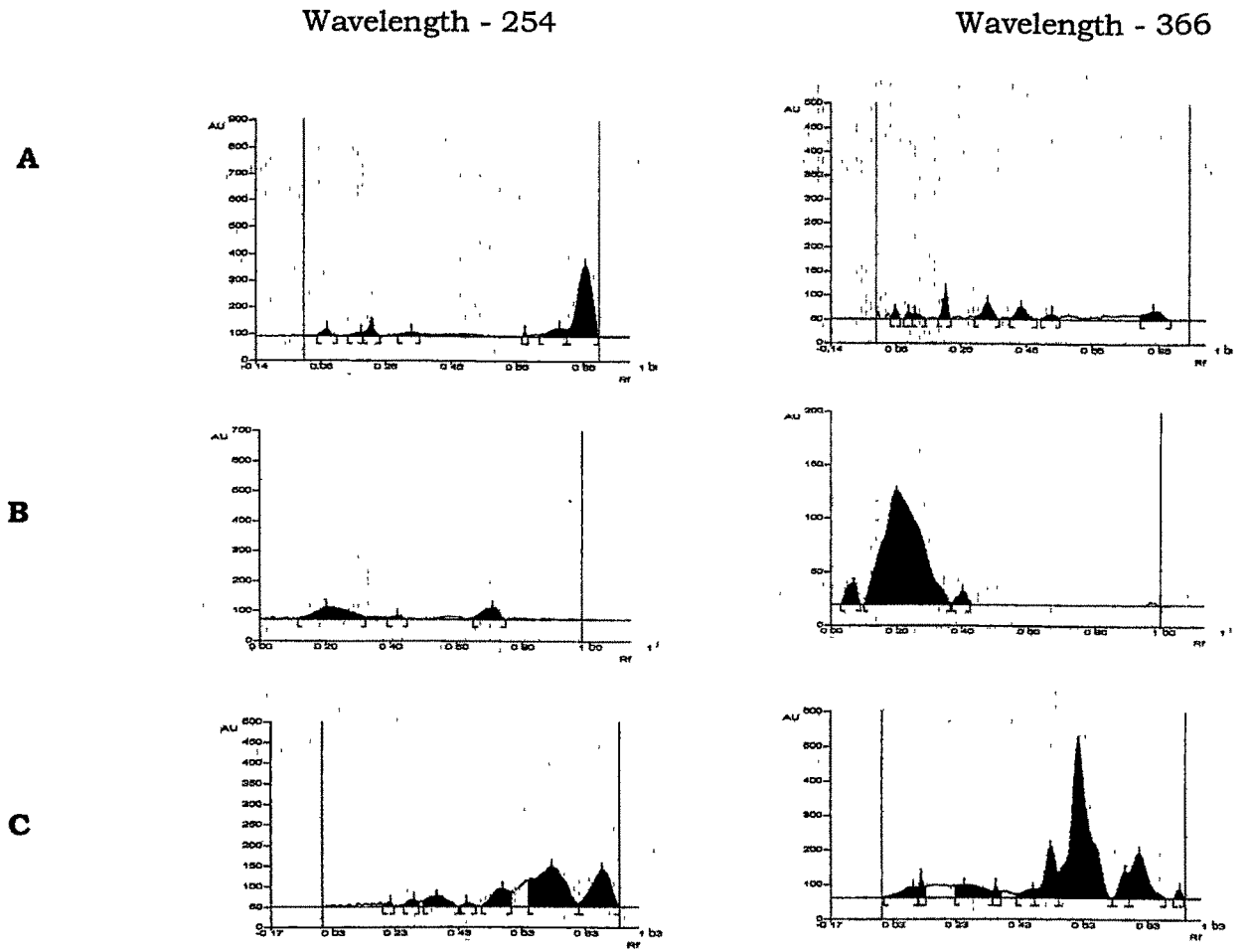


Fig. 3.53. TLC chromatogram of bioactive fraction of *S.indicus*. Solvent systems as in Table 3.24.

3.5.6 HPTLC fingerprint profile of petroleum ether extract of *S. indicus*

Solvent system 1: Petroleum ether extract resoluted in solvent system; hexane: ethyl acetate (9:1). Scanning at wavelength 254 nm revealed ten clearly separated spots. R_f of the separated compounds were 0.05, 0.08, 0.27, 0.33, 0.42, 0.52, 0.76, 0.85 and 0.92 with maximal absorbance at wavelength 207, 617, 270, 700, 256, 700, 624, 266, 270 and 269 nm. Compounds having maximum percentages were located at R_f 0.42 and 0.76 with respective percentages of 53.41 and 24.30 %.

Scanning of plate at wavelength 366 showed presence of ten components. R_f of these compounds were 0.06, 0.19, 0.24, 0.31, 0.39, 0.48, 0.59, 0.70, 0.73 and 0.92 with maximal absorbance at wavelength 411, 700, 489, 585, 583, 581, 700, 266, 263 and 268 nm. Four components were having considerable percentages and were located at R_f 0.06, 0.31, 0.39 and 0.59 with respective percentages 13.49, 18.69, 42.35 and 10.83 (Table 3.25, Fig 3.54).

Solvent system 2: Another solvent system used to resolute remaining components from petroleum ether fraction was; toluene: ethyl acetate (7:3). Plate when subjected to scan at 254 nm revealed the presence of seven compounds. R_f of these compounds were 0.19, 0.26, 0.36, 0.60, 0.84, 0.87 and 0.96, with λ_{max} 607, 633, 606, 607, 256, 700 and 264. Three compounds located at R_f 0.36, 0.84 and 0.96 shared major percentages among all i.e. 10.68, 54.87 and 18.11respectively.

Scanning of plate at 366 nm showed six clearly separated compounds. R_f of these components were 0.34, 0.45, 0.58, 0.79, 0.85 and 0.94 with λ_{max} 601, 558, 345, 369, 289 and 274 respectively. Compound located at R_f. 0.79 was found to have major share among all i.e. 81.75 % (Table 3.25, Fig 3.54).

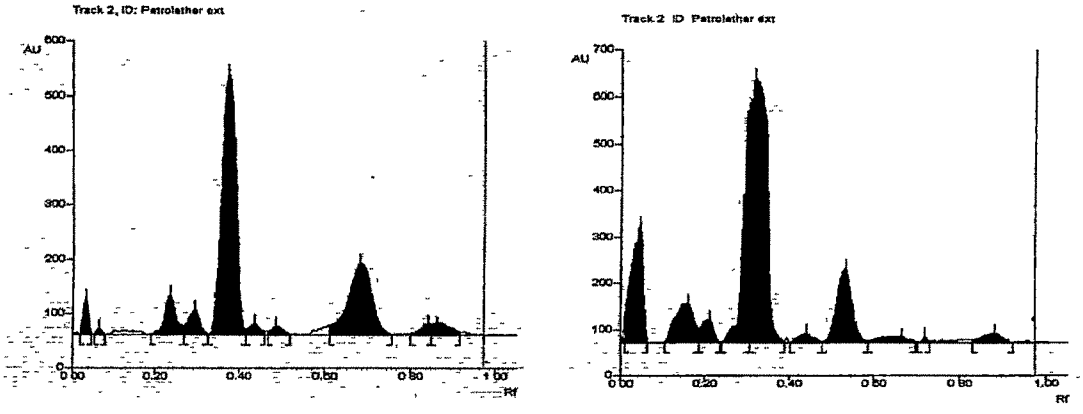
Table 3.25: HPTLC fingerprint profile of petroleum ether extract of *S. indicus*.

Scanned at	Solvent system 1			Solvent system 2		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.05	207	2.91	0.19	607	2.65
	0.08	617	0.43	0.26	633	4.48
	0.27	270	6.57	0.36	606	10.68
	0.33	700	4.24	0.60	607	8.37
	0.42	256	53.41	0.84	256	54.87
	0.46	700	1.74	0.87	-	0.85
	0.52	624	1.46	0.96	264	18.11
	0.76	266	24.30			
	0.85	270	1.67			
	0.92	269	3.26			
366 nm	0.06	411	13.49	0.34	601	4.36
	0.19	700	7.32	0.45	558	1.42
	0.24	489	2.68	0.58	345	6.56
	0.31	585	18.69	0.79	369	81.75
	0.39	583	42.35	0.85	289	1.99
	0.48	581	1.22	0.94	274	3.91
	0.59	700	10.83			
	0.70	266	1.56			
	0.73	-	0.24			
	0.92	268	1.62			

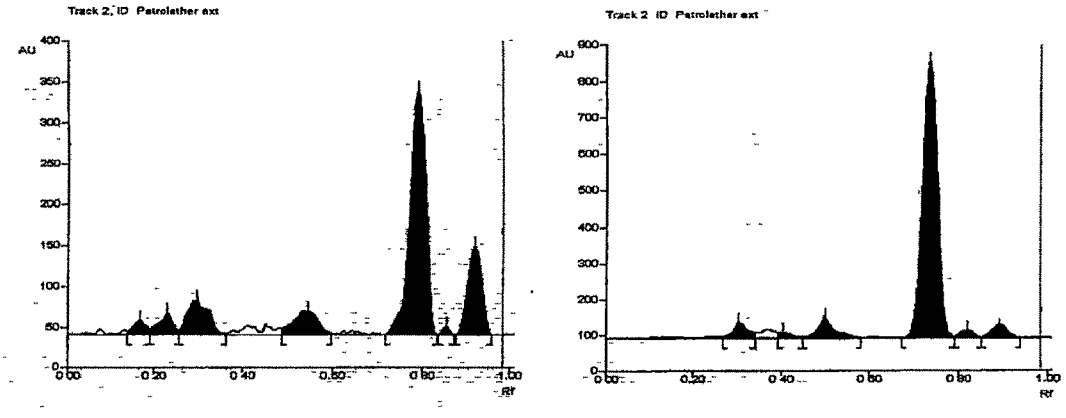
Solvent system 1: Hexane: ethyl acetate (9:1)

Solvent system 2: Toluene: ethyl acetate (7:3)

Wavelength 254 **Wavelength 366**



Solvent system 1



Solvent system 2

Fig 3.54: Chromatograms of petroleum ether extract of *S. indicus*.

Solvent system 1: Hexane: ethyl acetate (9:1)

Solvent system 2: Toluene: ethyl acetate (7:3)

3.5.7 HPTLC finger print profile of methanol extract of *C. pareira.*

Solvent system 1: Plate spotted with methanol extract of *C. pareira* was first run into solvent system, hexane: chloroform: methanol (4:5:1). Scanning of plate at 254 nm revealed presence of eight spots with R_f 0.22, 0.31, 0.39, 0.52, 0.65, 0.70, 0.85 and 0.95. These compounds had λ_{max} 200, 520, , 524, 700, 694, 203, 524 and 524 respectively. Respective percentages of these compounds were 16.68, 6.42, 5.00, 14.01, 28.11, 6.89, 11.00 and 11.89.

Scanning at 366 nm of this plate revealed presence of 7 spots. R_f of these compounds were 0.8, 0.14, 0.22, 0.30, 0.35, 0.90 and 0.95 with λ_{max} at 700, 700, 520, 524, 263, 268 and 694 nm respectively. Compounds located at R_f 0.22, 0.30, 0.35 and 0.90 were having substantiate proportion only i.e. 18.36, 20.25, 10.94 and 36.72 %, respectively.

Solvent system 2: Second solvent system used to resolute compounds from methanol extract was n-butanol: glacial acetic acid: water (6:2:2). Densitometric scanning at 254 nm revealed presence of six compounds. R_f of these compounds were 0.08, 0.18, 0.35, 0.46, 0.59 and 0.73 with maximal absorbance at wavelength 200, 202, 233, 200, 204 and 200 nm, respectively. Respective percentages of these compounds were 4.39, 19.82, 27.82, 14.29, 24.16 and 9.52 %.

Scanning at 366 nm with fluorescence mode reveled presence of 10 compounds with R_f 0.08, 0.18, 0.30, 0.39, 0.49, 0.57, 0.63, 0.70, 0.82 and 0.89. Respective wavelength maxima of these compounds were at 200, 201, 200, 200, 200, 203, 200, 200, 200, and 200 nm. Compounds with major percentages were 0.18 (20.55%), 0.30 (19.88%), 0.39 (11.90%), 0.49 (20.16%) and 0.57 (14.57%).

3.5.8 HPTLC finger print profile of alkaloidal fraction of *C. pareira.*

Solvent system 1: Plate spotted with alkaloidal fraction of C. pareira was first run into solvent system, hexane: chloroform: methanol (4:5:1). Scanning of plate at 254 nm revealed presence of eight spots with R_f 0.16, 0.20, 0.31, 0.39, 0.51, 0.65, 0.68 and 0.89. These compounds had λ_{max} 203, 200, 500, 694, 523, 524, 524 and 700 respectively. Respective percentages of these compounds were 3.25, 3.81, 5.00, 6.11, 22.81, 30.95, 12.85 and 15.21%.

Scanning at 366 nm of this plate revealed presence of 4 spots. R_f of these compounds were 0.06, 0.15, 0.23 and 0.30 with λ_{max} at 700, 700, 520 and 700 nm, respectively. Respective share of these compounds was, 22.16, 19.60, 33.08 and 25.16 %.

Solvent system 2: Second solvent system used to resolute compounds from alkaloidal fraction was, n-butanol: glacial acetic acid: water (6:2:2). Densitometric scanning at 254 nm revealed presence of seven compounds. R_f of these compounds were 0.08, 0.17, 0.33, 0.41, 0.46, 0.59 and 0.75 with maximal absorbance at wavelength 200, 200, 200, 200, 200, 203 and 203 nm, respectively. Respective percentages of these compounds were 5.24, 18.43, 13.73, 12.35, 10.29, 34.85 and 5.12 %.

Scanning at 366 nm with fluorescence mode reveled presence of 8 compounds with R_f 0.07, 0.20, 0.28, 0.36, 0.47, 0.60, 0.71 and 0.79. Respective wavelength maxima of these compounds were at 200, 200, 201, 203, 204, 200, 200 and 203 nm. Compounds with major percentages were 0.20 (18.52%), 0.47 (28.98%) and 0.60 (31.43%).

3.5.9 HPTLC fingerprint profile of Methanol extract of *C. orchioides*

Solvent system 1: Methanol extract of ***C. orchioides*** applied on TLC plate and resoluted in solvent system; toluene: ethyl acetate (7:3) and detected at 254 and 366 wavelength. Plate when scanned at 254 showed presence of six compounds. R_f of these compounds were 0.03, 0.09, 0.17, 0.34, 0.54 and 0.99 with λ_{max} 297, 409, 700, 700, 700 and 263 respectively. Major compound calculated from its area was found having R_f 0.03 with 76.18 % and other at R_f 0.99 having 16.55 %, rest other compounds had percentage less than 3%.

Plate when scanned at 366 nm with fluorescence mode it showed resolution of nine compounds. R_f of these compounds were 0.06, 0.12, 0.21, 0.34, 0.36, 0.38, 0.43, 0.51 and 0.76 with λ_{max} 289, 306, 200, 292, 290, 288, 200, 288 and 700, respectively. Major compounds calculated from their areas were located at R_f 0.06, 0.12, 0.21, 0.34 and 0.76 with 11.70, 11.52, 16.42, 20.93 and 21.55 % respectively (Table 3.28, Fig 3.57).

Solvent system 2: Methanol extract of ***C. orchioides*** subjected to resolute in solvent system; ethyl acetate: methanol: water (100: 13.5: 10). Plate when scanned at 254 nm using absorbance mode gave nine spots at R_f 0.13, 0.16, 0.24, 0.31, 0.39, 0.44, 0.49, 0.58 and 0.88 with λ_{max} 289, 200, 290, 286, 288, 200, 700, 200 and 700, respectively. Percentages of these compounds calculated from areas were found to be 51.32, 2.75, 12.24, 10.47, 11.74, 3.60, 2.23, 2.70 and 2.93, respectively.

Plate when subjected to scanning at 366 nm using fluorescence mode gave seven compounds at R_f 0.05, 0.16, 0.30, 0.42, 0.59, 0.74 and 0.95 with λ_{max} 700, 200, 200, 200, 200, 200 and 200 respectively. Major compounds were located at R_f 0.16, 0.30 and 0.95 with 11.30, 21.99 and 49.82% (Table 3.28, Fig 3.57).

Solvent system 3: The solvent system used was n-butanol: glacial acetic acid (6:2:2). Scanning plate at 254 nm showed resolution of eight

compounds. R_f of these compounds were 0.12, 0.20, 0.41, 0.69, 0.74, 0.82, 0.91 and 0.94 with λ_{max} 700 200, 286, 200, 200, 200, 200 and 263 respectively. A compound having major percentages was at R_f 0.41 with 68.46 %. Rests of the compounds were having lesser proportions.

Plate when subjected to scanning at 366 nm using fluorescence mode gave seven compounds at R_f 0.05, 0.16, 0.30, 0.42, 0.59, 0.74 and 0.95 with λ_{max} 700, 200, 200, 200, 200, 200 and 200 respectively. Major compounds were located at R_f 0.16, 0.30 and 0.95 with 11.30, 21.99 and 49.82% (Table 3.28, Fig 3.57).

Table 3.26: Finger print profile of methanol extract of *C. pareira* Linn.

Scanned at	Solvent system 1			Solvent system 2		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.22	200	16.68	0.08	200	4.39
	0.31	520	6.42	0.18	202	19.82
	0.39	524	5.00	0.35	233	27.82
	0.52	700	14.01	0.46	200	14.29
	0.65	694	28.11	0.59	204	24.16
	0.70	203	6.89	0.73	200	9.52
	0.85	524	11.00			
	0.95	524	11.89			
366 nm	0.8	700	3.12	0.08	200	4.01
	0.14	700	6.82	0.18	201	20.55
	0.22	520	18.36	0.30	200	19.88
	0.30	524	20.25	0.39	200	11.90
	0.35	265	10.94	0.49	200	20.16
	0.90	263	36.72	0.57	203	14.57
	0.95	694	3.79	0.63	200	4.95
				0.70	200	3.02
				0.82	200	0.42
				0.89	200	0.55

Solvent system 1: Hexane: Chloroform: Methanol (4:5:1)
Solvent system 2: n-Butanol: Glacial acetic acid: water (6:2:2).

Table 3.27: HPTLC finger print profile of alkaloidal fraction of *C. pareira*.

Scanned at	Solvent system 1			Solvent system 2		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.16	203	3.25	0.08	200	5.24
	0.20	200	3.81	0.17	200	18.43
	0.31	500	5.00	0.33	200	13.73
	0.39	694	6.11	0.41	200	12.35
	0.51	523	22.81	0.46	200	10.29
	0.65	524	30.95	0.59	203	34.85
	0.68	524	12.85	0.75	203	5.12
	0.89	700	15.21			
366 nm	0.06	700	22.16	0.07	200	4.94
	0.15	700	19.60	0.20	200	18.52
	0.23	520	33.08	0.28	203	6.00
	0.30	700	25.16	0.36	201	5.05
				0.47	204	28.98
				0.60	200	31.43
				0.71	200	4.21
				0.79	203	0.87

Solvent system 1: Hexane: Chloroform: Methanol (4:5:1)

Solvent system 2: n-Butanol: Glacial acetic acid: water (6:2:2).

Table 3.28: TLC finger printing profile of methanol extract of *C. orchioides*.

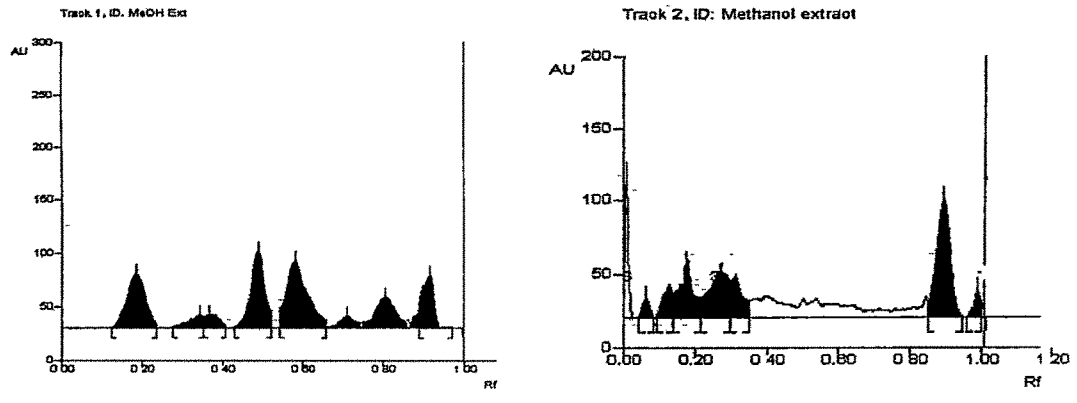
Scanned at	Solvent system 1			Solvent system 2			Solvent system 3		
	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %	Rf	λ_{max}	Relative %
254 nm	0.03*	297	76.18	0.13	289	51.32	0.12	700	2.16
	0.09	409	1.63	0.16	200	2.75	0.20*	200	1.47
	0.17	700	3.02	0.24*	290	12.24	0.41*	286	68.46
	0.29	700	1.68	0.31*	286	10.47	0.69	200	7.40
	0.54	700	0.95	0.39*	288	11.74	0.74	200	9.56
	0.99*	263	16.55	0.44*	200	3.60	0.82	200	6.02
				0.49	700	2.23	0.91*	200	2.28
				0.58	200	2.70	0.94	263	2.64
				0.88	700	2.93			
366 nm	0.06	289	11.70	0.05*	700	4.75	0.05*	700	4.75
	0.12*	306	11.52	0.16*	200	11.30	0.16	200	11.30
	0.21	200	16.42	0.30	200	21.99	0.30	200	21.99
	0.34	292	20.93	0.42	200	6.43	0.42	286	6.43
	0.36	290	2.70	0.59	200	3.59	0.59	200	3.59
	0.38	288	3.91	0.74*	200	2.12	0.74	200	2.12
	0.43*	200	4.42	0.95	200	49.82	0.95	200	49.82
	0.51*	288	6.86						
	0.76*	700	21.55						

Solvent system 1. Toluene/Ethyl acetate (7:3 v/v);

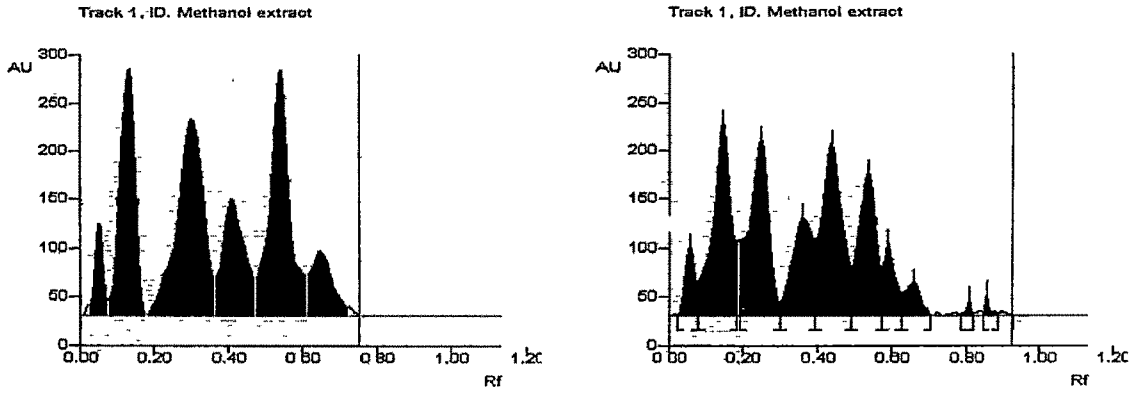
Solvent system 2. Ethyl acetate/Methanol/Water (10:1.35:1.00 v/v);

Solvent system 3. n-butanol/glacial acetic acid/water (6:2:2 v/v).

Wavelength 254 **Wavelength 366**



Solvent system- 1



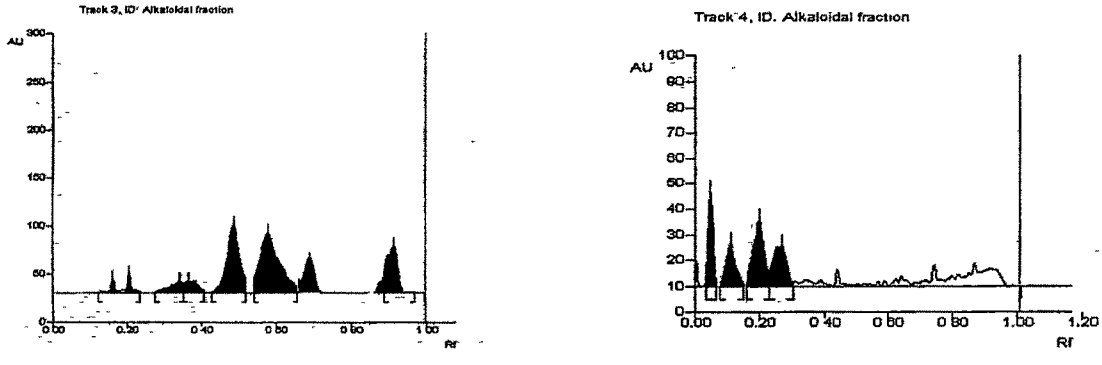
Solvent system- 2

Fig 3.55: Chromatograms of methanol extract of *C. pareira.*

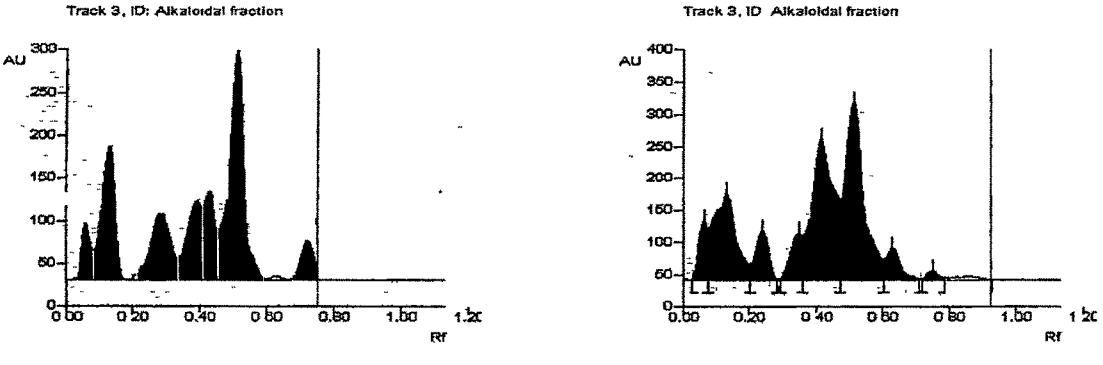
Solvent system 1: Hexane: Chloroform: Methanol (4:5:1)

Solvent system 2: n-Butanol: Glacial acetic acid: water (6:2:2).

Wavelength 254 **Wavelength 366**



Solvent system- 1



Solvent system- 2

Fig 3.56: Chromatograms of alkaloidal fraction of *C. pareira.*

Solvent system 1: Hexane: Chloroform: Methanol (4:5:1)

Solvent system 2: n-Butanol: Glacial acetic acid: water (6:2:2).

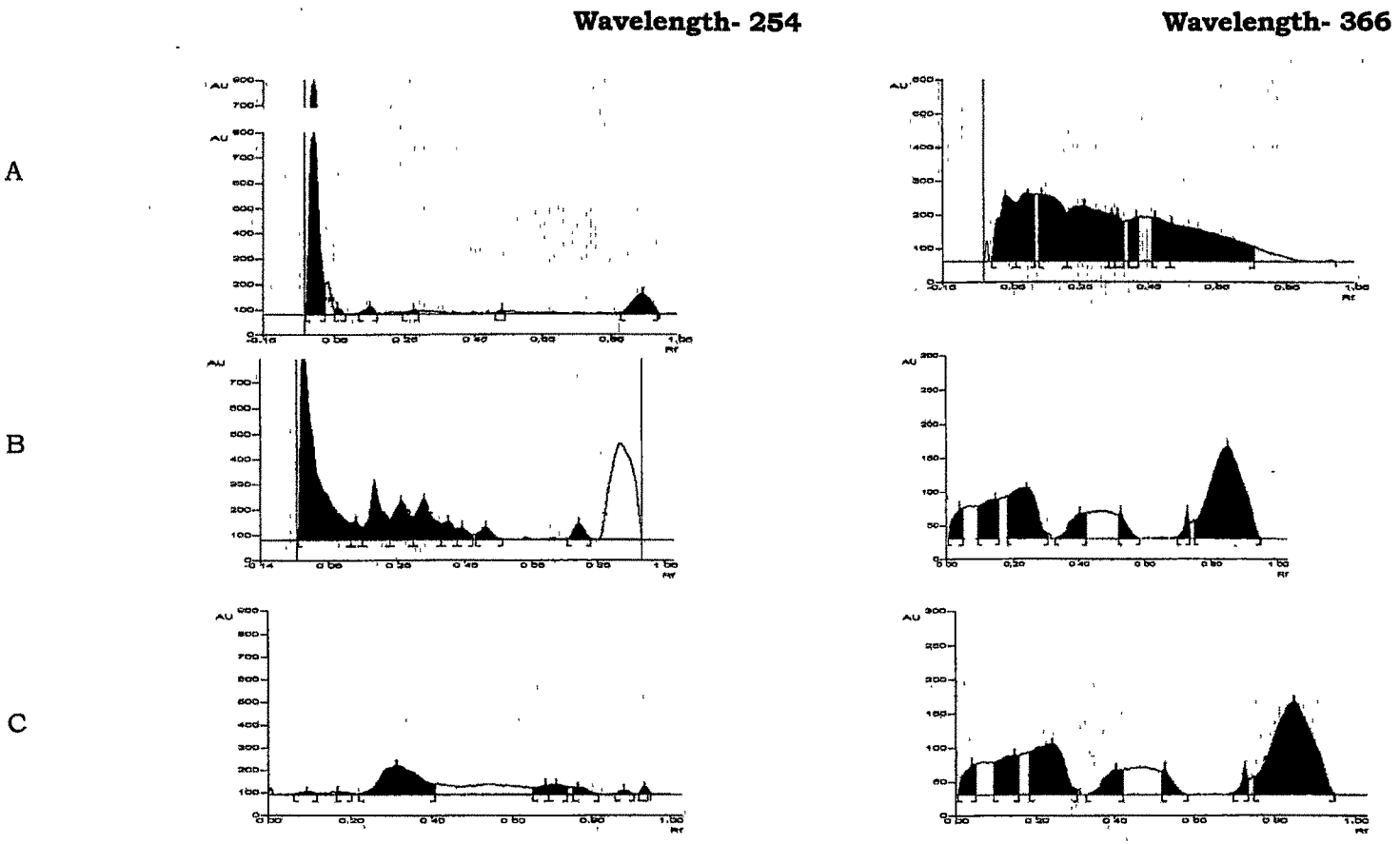


Fig 3.57: Chromatograms of methanol extract of *C. orchioides*.

A- Solvent system 1. Toluene/Ethyl acetate (7:3 v/v);

B- Solvent system 2. Ethyl acetate/Methanol/Water (10:1.35:1.00 v/v);

C- Solvent system 3. n-butanol/glacial acetic acid/water (6:2:2 v/v).

3.6 ISOLATION AND CHARACTERIZATION OF COMOUNDS FROM BIOACTIV EEXTRACTS:

Different bioactive extracts from selected plants were subjected to isolation of compounds by applying different methods including fractionation of the extracts and column chromatographic techniques. Data pertaining to physiochemical and spectral studies were recorded.

1. Compounds from flower heads of *S. indicus*

Compound-S-1

The compound S-1 isolated from petroleum ether extract. The unsaponifiable matter was separated and fractionated into different solvents. Benzene fraction yielded compound **S-1**.

Description: White sticky powder.

Solubility: Soluble in hexane, benzene, ethyl acetate,

m.p.- 60-65 ^{0}C

Elemental analysis: C= 70.23, H= 8.74, O= 11.69

Emperical formula: $C_8H_{12}O$

IR: 3449 (OH group, H bending), 2922, 2851 (Methylene –CH stretch), 2362, 1595, 1466 (-CH bend), 1383 (Tri methyl), 1351 (Weak –CH_3), 1015, 670 (-OH bend), 553.

1H-NMR: 2.38, 2.35, 2.31, 1.63, 1.60, 1.35, 1.25, 1.15, 0.98, 0.89, 0.85,

Mass: B/P- 95 m/z.

UV Analysis: 326.5 and 245.5 nm

Compound- S-2

The compound S-2 was isolated from unsaponifiable matter of petroleum ether extract. Benzene fraction yielded S-2 by column chromatography.

Description: White free flowing powder.

Solubility: Soluble in hexane, ethyl acetate, chloroform.

m.p.- 55-58 ^{0}C

Elemental analysis: C= 78.17, H= 11.67 O= 4.64.

Emperical formula: $C_{22}H_{40}O$

IR: 3436 (OH group, H bending), 2940 (Aliphatic –CH), 2372 (-CH stretch), 1945, 1598, 1460, 1378, 1261 (Primary –OH), 1192, 1098, 1058 (Primary alcohol –CO stretch), 736, 700, 625 (-OH bend).

1H-NMR: 5.36, 5.34, 5.14, 5.11, 2.05, 5.02, 3.52, 2.27, 2.23, 2.00, 1.97, 1.86, 1.83, 1.73, 1.69, 1.67, 1.65, 1.53, 1.48, 1.44, 1.43, 1.40, 1.28, 1.25, 1.21, 1.19, 1.16, 1.16, 1.14, 1.13, 1.08, 1.07, 1.03, 1.01, 0.96, 0.93, 0.91, 0.85, 0.84, 0.83, 0.82, 0.80, 0.78, 0.70, 0.68, 0.53, 0.07.

Mass: B/P- 82.

UV Analysis (Hexane): 378, 374, 280, 212 nm.

Compound-S-3

The compound S-3 was isolated from petroleum ether extract by fractionating it into hexane, benzene, chloroform, ethyl acetate. Hexane fraction yielded compound S-3 by column chromatography.

Description: White amorphous free flowing powder.

Solubility: Soluble in hexane, ethyl acetate, chloroform.

m.p.- 72- 75 ^{0}C

Elemental analysis: C= 82.38, H= 13.96, O= 1.39.

Emperical formula: $C_{80}H_{160}O$

IR: 3445, 2920, 2850 (-CH stretch), 2656, 2373, 2340 (Aliphatic –CH), 1746 (Ester or alkyl carbamate), 1598 (Carboxylate salt), 1466, 1377, 1262, 1097, 888, 801, 724 (Methylene, $-(CH_2)_n$ rocking).

1H-NMR: 1.54, 1.25, 0.90, 0.88, 0.86, 0.07,

Mass: B/P- 95.

UV Analysis: 259, 220, 241 nm.

Compound- S-4

The compound S-4 was isolated from petroleum ether fraction of methanol extract by column chromatography.

Description: White amorphous powder.

Solubility: Soluble in hexane, ethyl acetate, chloroform.

m.p.- 80-85 ^{0}C

Elemental analysis: C= 84.06, H= 10.97, O= 1.06.

Emperical formula: $C_{22}H_{35}O$

IR: 3420 (-OH group), 2920, 2849 (-CH stretch), 2363, 1595, 1466, 1381, 1351 (Methyl –CH stretch and bend), 1019, 723 (long linear $-CH_2$ chain), 669, 523.

1H-NMR: 1.55, 1.35, 1.25, 1.15, 0.88.

Mass: B/P- 89 m/z.

UV Analysis: 232 nm.

Compound- S-5

Source: White free flowing powder.

Solubility: Soluble in hexane, ethyl acetate, chloroform.

m.p.- 75-80 ^0C

Elemental analysis: C= 74.72, H= 12.79, O= 9.93.

Emperical formula: $C_{10}H_{20}O$

IR: 3450 (-OH group), 2952, 2850(-CH stretch), 2363, 1615, 1466, 1351(Methyl –CH stretch and bend), 723 (long linear $-CH_2$ chain).

1H-NMR: 2.37, 2.35, 2.32, 1.66, 1.63, 1.61, 1.25, 1.06, 0.90, 0.88, 0.86, 0.07,

Mass: N.A.

UV Analysis: 237 nm.

Compound- S-6

The compound S-6 was isolated from Petrol ether fraction of methanol extract of *S.indicus* by column chromatography.

Description: Yellowish amorphous powder.

Solubility: Soluble in benzene, ethyl acetate,

m.p.- 240 ^0C

Elemental analysis: C=71.01, H= 7.53, O= 18.53.

Emperical formula: C_4H_5O

IR: 3400 (Hydroxyl group –H bend), 2928, 2848 (Methylene –CH stretch), 1684, 1652, 1459, 1436, 1367 (Methyl –CH stretch and bend), 1166, 1023, 971, 961, 888, 799.

1H-NMR: 2.76, 2.66, 2.60, 2.59, 2.58, 2.57, 2.56, 1.25, 1.03, 1.00, 0.86, 0.83, 0.80, 0.77, 0.69, 0.68

Mass: B/P- 396 m/z.

UV analysis: 210 nm.

Compound-S-7

Source: Petroleum ether fraction of methanol extract.

Description: Yellowish free flowing powder.

Solubility: Soluble in hexane, ethyl acetate, chloroform.

m.p.- 81-82 ^{0}C

Elemental analysis: C= 77.64, H= 12.42, O= 7.19.

Emperical formula: $C_{14}H_{27}O$

IR: 2919, 2850 (Methyl –CH stretch), 2373, 2342, 1705 (Ketonic carbonyl group), 1597, 1467, 1414 (-CH bending vibrations), 1351, 1299 (-CH bending vibrations), 722 (Methylene –(CH_2)n rocking), 546.

1H-NMR: 2.38, 2.35, 2.32, 1.66, 1.63, 1.61, 1.30, 1.25, 0.90, 0.88, 0.86.

Mass: B/P- 95

UV Analysis (Hexane): 244 nm

Compound-S-8

Source: Bioactive fraction by fractionation method.

Description: Yellowish amorphous powder.

Solubility: Soluble in benzene, ethyl acetate,

m.p.- 110-115 ^{0}C

Elemental analysis: C= 63.36, H= 8.63, O= 3.85.

Emperical formula: $C_{22}H_{35}O$

IR: 3437 (-OH group), 2962, 2920, 2850 (-CH stretch), 2732, 2363, 1595, 1469, 1382, 1351, 1263, 1098, 1023, 801, 721 (Methylene –(CH_2)n rocking).

1H-NMR: 1.57, 1.35, 1.25, 1.15, 0.88, 0.086, 0.069, 0.0512, 0.00.

Mass: B/P- 154 m/z.

UV Analysis: 232 nm

2. Compounds from roots of *C. pareira*

Compound C-1

Isolated from methanol extract after fractionating it into different solvents.

Description: White sticky compound.

Solubility: Soluble in hexane, ethyl acetate, chloroform.

m.p.- 75-80 ^{0}C

Elemental analysis: C= 74.72, H= 12.79, O= 9.93.

Empirical formula: $C_{10}H_{20}O$

IR: 3450 (-OH), 2923, 2854 (-CH stretch), 2373, 2340, 2131, 1740 (-CO group), 1567, 1446, 1357(-CH bending vibrations), 1174, 1109, 720

1H-NMR: 7.26, 5.70, 5.34, 4.02, 3.67, 3.41, 2.85, 2.66, 2.52, 2.42, 2.37, 2.35, 2.32, 2.30, 2.28, 2.02, 2.00, 1.63, 1.61, 1.30, 1.25, 0.90, 0.88, 0.86, 0.68, 0.08,

Mass: Not interpreted.

UV Analysis: 240 nm

Compound C-2

Source: Methanol extract of roots of *C.pareira.*

Description: Cream colored free flowing powder.

Solubility: Soluble in chloroform and methanol.

m.p.- 210-211 ^{0}C

Elemental analysis: C= 47.02, H= 6.38, O= 37.57, N= 3.82

Empirical formula: $C_{14}H_{23}NO_8$

IR: 3729 (-NH), 3329 (-OH), 2357, 1596, 1384, 1270, 1236, 1179, 1135, 1045, 934, 899, 869, 775, 658, 592

1H-NMR: 7.26, 1.56, 1.25, 0.85.

Mass: Not interpreted.

UV Analysis: 293, 270, 244

3. Compound from *C. orchioides*

Compound-O

This compound was isolated from the ethyl acetate fraction was methanol extract by column chromatography.

Description: Buff white free flowing amorphous powder.

Solubility: ethyl acetate and methanol.

m.p.- 110-111 ^{0}C.

Elemental analysis: N.A.

Empirical formula: N.A.

IR: 3387, 2923, 2853, 2372, 1596, 1463, 1375, 1260, 1166, 1071, 1023, 889, 800, 719, 622.

1H-NMR: N.A.

Mass: N.A.

UV Analysis: 263, 285 nm.