Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

ANTHELMINTIC AND FREE-RADICAL SCAVENGING POTENTIAL OF VARIOUS FRACTIONS OBTAINED FROM FOLIAR PARTS OF *GLINUS OPPOSITIFOLIUS* (LINN.) DC

BHASKAR DUTTA[1], MITALI GHOSAL[2], PRANABESH CHAKRABARTY[1], PALASH MANDAL[2]*

[1]Bengal School of Technology, Sugandha, Delhi Road, Hooghly - 712102, West Bengal, India, [2]Plant Physiology and Pharmacognosy Research Laboratory, Department of Botany, University of North Bengal, Siliguri 734013. Email: nbubotanypalash@rediffmail.com

Received: 16 July 2012, Revised and Accepted: 29 Aug 2012

ABSTRACT

The anthelmintic and DPPH radical scavenging activity of different solvent fractions obtained from edible leafy vegetable *Glinus oppositifolius* was investigated and their association with polyphenol contents was evaluated. Leaves were extracted with aqueous-methanol (4:1) by soxhelation. The extract was successively fractionated with increasing solvent polarity. Total phenolics and flavonoid contents of different fractions were determined by Folin-Ciocalteu and aluminium chloride based reagents, respectively. Free radical scavenging activity was assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. *In vitro* anthelmintic activity was carried out by aquarium worm, *Tubifex tubifex.* Both, pet ether and diethyl ether fractions were observed to have higher level of phenolic content (32.12±0.83 and 47.82±1.04 mg gallic acid equivalent/g respectively) and flavonoids content (10.45±0.94 and 4.09±0.52 mg quercetin equivalent/g respectively) with potential free radical scavenging activity (IC_{50}=0.267±0.0006 mg/ml and IC_{50}=0.176±0.0015 mg/ml respectively). The anthelmintic activity of pet ether, diethyl ether and ethyl acetate fractions were found to be more effective than that of reference standard, piperazine citrate. Highest paralyzing activity was obtained by pet ether fraction whereas the death potency was optimum in case of ethyl acetate. The anthelmintic activity of *G. oppositifolius* leaves were significantly correlated with polyphenolic contents and free radical scavenging potential. Pet ether, diethyl ether and ethyl acetate fractions obtained from hydro alcoholic extract of *G. oppositifolius* leaves are rich in phenolic antioxidant and elicited significant anthelmintic activity, comparable to that of standard. Further studies are required to isolate the active principles responsible for anthelmintic activity as well as support the endogenous antioxidant defense system.

Keywords: DPPH, Total phenol, Total flavonoid, Anthelmintic, *Glinus oppositifolius*

INTRODUCTION

Helminth infections are commonly found in community and being recognized as cause of acute and chronic illness among the human beings as well as cattle. Helmenthiasis is highly prevalent particularly in third world countries [1] and is widespread globally with poor personal and environmental hygiene. Human gastrointestinal tract is the abode of many helminthes, some also live in tissues or their larvae may migrate into different tissues. In some cases the toxic, antigenic substances secrets from the parasites' egg may destroy the cells of host tissue, stimulates inflammatory reaction and impairs antioxidant defense system [2-3]. The sophisticated antioxidant system may help to protect against free radical induced cellular damages, co-infection and life threatening diseases. Helminthes also harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia [4]. These worm diseases cause serious morbidity and affect population in endemic areas [5].

Anthelmintics are the substances that either kill (Vermicide) or expel (Vermifuge) intestinal parasitic worms. They may interfere with parasite's carbohydrate metabolism, inhibit respiratory enzyme, block neuromuscular action or make them susceptible to destruction by host's macrophages. Now a day, the gastrointestinal helminthes become resistant to currently available synthetic anthelmintics due to indiscriminant use of these types of drugs [6-7]. Moreover, residual toxicity, adverse drug reaction, high costs are the problems associated with these agents. Plant derived drugs serve as prototype to develop more effective and less toxic medicines. The plants are known to provide a rich source of botanical anthelmintics [8-10]. A number of medicinal plants have been used to treat parasitic infections in men and animals [11-12]. Unlike synthetic anthelmintics, plant based anthelmintics with different mode of actions could be able to prevent the development of resistance. Also several studies have claimed that some synthetic polyphenolics like niclosamide, bithionol etc[13] and natural polyphenolics like tannin may act as anthelmintics [14]. At the same time, phenolic compound present in the medicinal plant has been reported to possess powerful antioxidant activity [15-16]. Therefore, there is a foremost need in treatment of helminthes diseases to develop newer, more effective and less toxic anthelmintics with high free radical scavenging potential that can minimize the impact of helminth injury.

A bitter leafy vegetable, Glinus oppositifolius (Linn.) DC, (Family: Molluginaceae) is known as 'Gima shak' in West Bengal, Assam and Bangladesh. The plant is highly esteemed in India, eaten occasionally on account of its stomachic, appetizer and antiseptic properties. G. oppositifolius is used to treat joint pain, inflammation, diarrhoea, intestinal parasites, fever, and malaria [17]. Hence, in this present study, we aimed to evaluate anthelmintic and free radical scavenging potential of partially purified extract of G. oppositifolius leaves, which may serve as new source of medicine.

MATERIALS AND METHODS

General

2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, Quercetin, aluminum chloride, potassium acetate, sodium carbonate, dimethyl sulfoxide (DMSO) were either purchased from Sigma Chemical (USA) or Himedia or Merck (Germany). All other chemicals and solvents were of analytical grade. The absorbance measurements were recorded using the double beam ultraviolet-visible spectrophotometer of Systronics, 2201.

Plant material

The leaves of *Glinus oppositifolius* (Linn.) DC were collected from Hooghly, West Bengal, India in July 2011. The taxonomic identification was done by Prof. A. P. Das, Taxonomy and Environmental Biology Laboratory, North Bengal University (NBU), Darjeeling, India. A voucher specimen (B/Chu/02) was deposited at laboratory herbarium, Taxonomy and Environmental Biology Laboratory, NBU, Darjeeling, India and identified against the accession number 9620 for the further references.

Preparation of extract

Plant was washed in tap water and 500gm of fresh leaves were extracted using methanol: water (4:1) in a Soxhlet apparatus for 24

h. The extract was concentrated to one fifth volume under a vacuum rotary evaporator. The extract thus obtained was finally fractionated with the use of solvent in increasing order of polarity which was shown in the flowchart (Figure 1).

Fractions encoded as PE-FC: Pet ether fraction; HEP-FC: Heptane fraction; DEE-FC: Diethyl ether fraction; EA-FC: Ethyl acetate fraction; CHL-FC: Chloroform fraction; BUT-FC: Butanol fraction, ACE-FC: Acetone fraction, MEOH-FC: Methanol fraction; AQU-FC: Aqueous fraction; LIP-FC: Lipid part fraction.

Animal selection

Aquarium worms, *Tubifex tubifex* (Annelida) were collected from the local market. The average sizes of the worms were 1-1.5 cm. The worm types were identified at P.G. Department of Zoology, NBU, West Bengal. The assay was performed on aquarium worm, *Tubifex tubifex* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings [18-20]. Because of easy availability, aquarium worms have been used widely for initial evaluation of anthelmintic compounds *in vitro*.

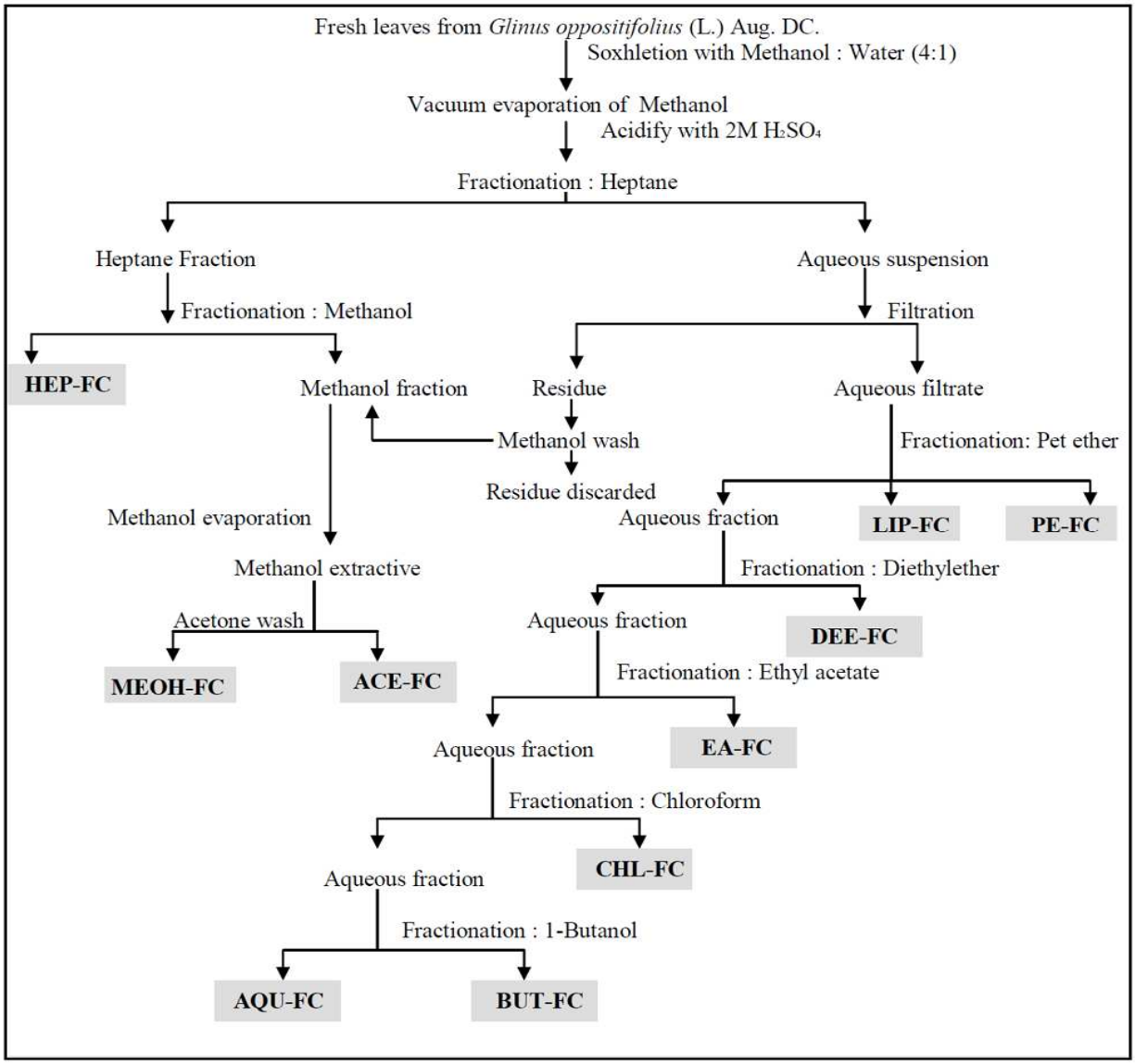


Fig. 1: Schematic diagram of fractionation process with *G. oppositifolius* leaf extract

Total phenolic contents (TPC)

Total phenolic contents of plant extracts were determined according to the standard protocol discussed previously [21]. 1ml of the each fraction was mixed in a test tube containing 1ml of 95% ethanol, 5ml of distilled water and 0.5ml of 50% Folin-Ciocalteu reagent. The resultant mixture was allowed to react for 5 min and 1ml of 5% Na_2CO_3 was added. It was mixed thoroughly and placed in dark for 1h. Finally the absorbance of colored reaction product was measured at 765nm against the reagent blank. The total phenolic content was expressed as mg of gallic acid equivalent per gram (mg GAE/gm) of dried extract.

Total flavonoid content (TFC)

The TFC were measured following a previously reported spectrophotometric method [22]. Briefly, extracts of each solvent fraction (1ml containing 0.1 mg/ml) were diluted with water (4ml) in a 10ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.3ml) was added to each volumetric flask; at 5 min, 10% $AlCl_3$ (0.3ml) was added; and then after 6 min, 1M NaOH (2ml) was added. Next, water (2.4ml) was added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm. The calibration curve was prepared by measuring absorbance of quercetin at various concentrations in methanol. The total flavonoid content in different

fractions was calculated as quercetin equivalent per gram (mg QE/gm) of extract.

Free radical scavenging assay

DPPH scavenging assay is one of the most preferred antioxidant method for determination of the free radical scavenging activity of plant material. Activity of the extract, based on the scavenging of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, was studied. 200µl of solvent fractions of different concentrations were taken in different test tubes and 2ml of DPPH (100µM/ml) solution was added to each test tube. The test tubes were then incubated for 30 minutes to complete the reaction. The absorbance of the solution was measured at 517nm using a spectrophotometer against blank[23] and the free radical scavenging activity was calculated in the form of IC_{50} value. The IC_{50} value is the concentration where 50% inhibition of free radicals occurs. The percentage (%) inhibition activity was calculated from the linear equation:

Percentage (%) inhibition = $[(A_0 – A_1)/A_0]$ x 100.

Where, A_0: absorbance of the control and A_1: absorbance of the extract or standard. Then percentage inhibitions were plotted against concentration and from the graph, IC_{50} was calculated.

***In vitro* anthelmintic activity**

The anthelmintic assay was carried out as per the method describe previously with minor modifications [24]. All the fractions of *G. oppositifolius* were dissolved in minimum amount of DMSO and then volume was adjusted to 10ml with distilled water. 10ml of formulation containing three different concentrations of each of fractions were prepared and a lump of worms were placed in 10ml solution containing petridish. All drugs and extract solutions were freshly prepared before starting the experiment. Time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously or dipped in warm water (50 °C). Time for death of worms was recorded after ascertaining that the worms were not moved when shaken and followed with fading away of their body colors. Piperazine citrate (10 mg/ml) was used as reference standard while distilled water with DMSO as control [25-26].

Statistical analysis

All assays were conducted in triplicate. The results were reported as mean values of three analysis and standard deviations (SD). Data were subjected to statistical analysis using the SPSS program, release 11.0 for Windows (SPSS CHICAGO IL USA). The one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) were employed by DSAASTAT ver. 1.022, (Andrea Onofri, Perugia, Italy) to study the effect of solvent and the differences between the means were deemed to be significant at $p<0.05$. Simple association between antioxidant variables were calculated as the Pearson Correlations. Principle Component analysis were performed by Statistic XL 1.8 to detect and visualize the structure in the relationship between anthelmintic activity and bioactive component of antioxidants.

RESULTS

Extractive value (EV)

Extractive values of different solvent fractions of *G. oppositifolius* leaves were given in Table 1. Among all fractions tested, AQU-FC was found to be maximal followed by BUT-FC.

Table 1: Extractive values, total phenolic and flavonoid content of different solvent fractions of *G. oppositifolius*

Solvent fraction	Extractive value wt(gm)	TPC mg GAE/gm of extract	TFC mg QE/gm of extract
PE-FC	0.081±0.002^{j}	32.12±0.83^{b}	10.446±0.94^{a}
DEE-FC	0.808±0.003^{i}	47.82±1.04^{a}	4.091±0.52^{b}
EA-FC	1.703±0.01^{f}	17.33±0.5^{c}	2.543±0.37^{d}
CHL-FC	0.926±0.06^{h}	5.99±0.53^{f}	0.655±0.03^{g}
ACE-FC	4.833±0.034^{c}	5.59±0.26^{f}	2.062±0.75^{e}
BUT-FC	4.921±0.045^{b}	13.51±0.57^{d}	3.805±0.71^{c}
MEOH-FC	1.17±0.021^{g}	11.05±0.36^{e}	1.655±0.59^{f}
AQU-FC	6.195±0.017^{a}	1.77±0.23^{g}	0.248±0.02^{h}
HEP-FC	1.794±0.012^{e}	5.64±0.6^{f}	1.965±0.44^{e}
LIP-FC	2.401±0.032^{d}	1.69±0.12^{g}	0.248±0.02^{h}

In each column means having different letter(s) are significantly ($P<0.01$) different as determined by DMRT. Each value represents Mean ± SD (n=3)

Total phenolic content (TPC)

On the basis of the results (Table 1), the DEE-FC was observed to possess highest amount of phenolics followed by PE-FC and EA-FC while LIP-FC was shown to be the least. The content of the total phenolics in fractions was determined by using the linear regression equation of the calibration curve ($y = 122.14x – 6.2663$, $r^2=0.9991$).

Total flavonoid content (TFC)

The results (Table 1) revealed that the PE-FC was found to contain the highest amount of flavonoids followed by DEE-FC, BUT-FC and EA-FC while the lowest amount of the same was observed in LIP-FC. The TFC of different fractions were determined by using the linear regression equation of the calibration curve ($y = 226.15x – 4.0941$, $r^2=0.9956$).

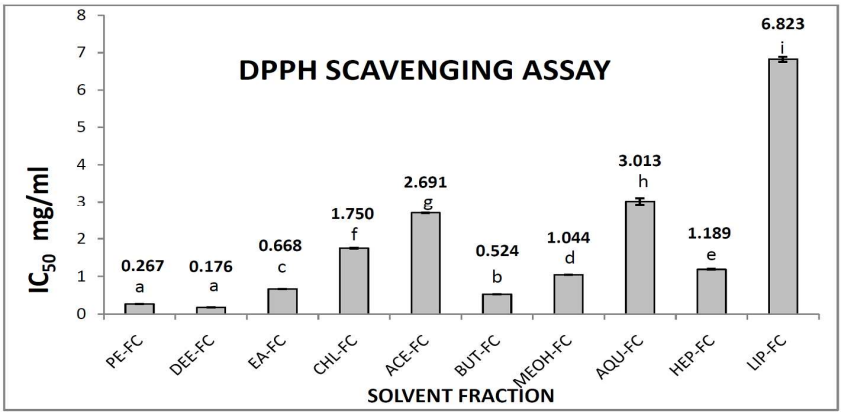


Fig. 2: DPPH scavenging activity of different solvent fractions of *G. oppositifolius*

Mean having different letter indicates significant ($P<0.01$) differences between different solvent fraction as determined by DMRT.

DPPH radical scavenging activity

The extract exhibited a dose dependent inhibition of free radicals through scavenging of DPPH. Different solvent fractions exhibited considerable free radical scavenging activity as indicated by their IC_{50} values which was shown in Fig 2. DEE-FC was shown to have a reliable IC_{50} value (0.176mg/ml) followed by PE-FC, BUT-FC and EA-FC.

Anthelmintic activity

Anthelmintics activity of different solvent fractions of *G. oppositifolius* was evaluated by using aquarium worm, *Tubifex tubifex*. Potency of the solvent fraction was found to be inversely proportional to the time taken for inhibition of spontaneous motility (paralysis) and death. The effects were comparable with that of standard piperazine citrate (10 mg/ml), (Table 2)

Table 2: Anthelmintic activity of different solvent fractions of *G. oppositifolius*

Solvent	Paralysis		Death	
	Duration (min)	CONC. (mg/ml)	Duration (min)	CONC. (mg/ml)
PE-FC	13.52±0.18^{k}	2.5	56.35±0.37^{q}	2.5
	8.85±0.90^{gh}	5	36.52±0.23^{lm}	5
	7.02±0.64^{e}	10	21.03±0.12^{g}	10
MEOH-FC	40.35±0.12^{q}	10	97.07±0.66^{w}	10
	18.35±0.29^{m}	15	47.03±0.35^{p}	15
	7.25±0.02^{ef}	20	19.83±0.44^{f}	20
LIP-FC	34.35±0.54^{p}	20	78.30±0.22^{v}	20
	8.07±0.77^{fg}	50	35.87±0.18^{l}	50
	2.05±0.45^{b}	100	6.12±0.04^{c}	100
EA-FC	9.00±0.17^{gh}	5	14.67±0.04^{e}	5
	3.25±0.32^{c}	10	6.92±0.18^{c}	10
	2.15±0.26^{b}	50	4.10±0.18^{b}	50
	0.38±0.27^{a}	100	1.50±0.23^{a}	100
DEE-FC	16.03±0.11^{l}	5	70.03±0.38^{t}	5
	10.65±0.07^{i}	10	38.12±0.43^{n}	10
	9.00±0.13^{gh}	20	21.72±0.24^{g}	20
CHL-FC	41.07±0.44^{q}	10	47.57±0.76^{p}	10
	24.63±0.29^{o}	20	37.33±0.84^{mn}	20
	12.17±0.75^{j}	40	21.58±0.75^{g}	40
BUT-FC	23.85±0.76^{o}	5	75.20±0.26^{u}	5
	5.62±0.55^{d}	25	26.75±0.61^{i}	25
	1.73±0.12^{b}	50	9.48±0.72^{d}	50
AQU-FC	18.97±0.19^{m}	25	62.52±0.87^{r}	25
	4.08±0.86^{c}	50	28.77±0.28^{j}	50
	1.65±0.76^{b}	100	8.77±0.12^{d}	100
ACE-FC	46.93±0.54^{r}	10	64.92±0.45^{s}	10
	19.03±0.73^{m}	25	32.78±0.33^{k}	25
	9.48±0.08^{h}	50	22.90±0.56^{h}	50
HEP-FC	Not paralyzed		Not dead	
piperazine citrate	22.07±0.12^{n}	10	45.50±0.38^{o}	10

In column time mean having different letter(s) are significantly (P< 0.05) different as determined by DMRT. Each value represents mean± SD (n=3)

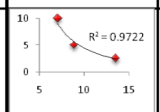
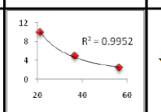
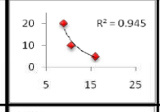
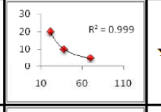
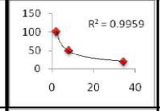
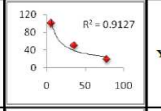
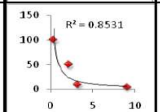
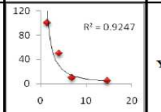
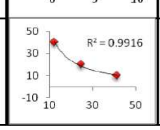
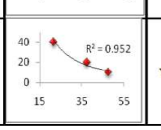
	PARALYSIS			DEATH		
SOLVENT FRACTION	Graphical Representation (x axis denote Time & y axis denote Concentration)	Kinetic equation.	Conc. (mg/ml) required for performing Piperazine Citrate (10mg/ml) equiv. bioactivity	Graphical Representation (x axis denote Time & y axis denote Concentration)	Kinetic equation.	Conc. (mg/ml) required for Performing Piperazine Citrate (10mg/ml) equiv. bioactivity
PE-FC		$Y=505.05x^{-2.056}$	0.89		$Y=728.18x^{-1.4}$	3.48
DEE-FC		$Y=2572.7x^{-2.269}$	2.36		$Y=756.6x^{-1.183}$	8.27
LIP-FC		$Y=155.39x^{-0.672}$	26.62		$Y=316.31x^{-0.69}$	33.26
EA-FC		$Y=48.567x^{-0.975}$	2.41		$Y=210.59x^{-1.39}$	1.03
CHL-FC		$Y=695x^{-1.13}$	21.06		$Y=7144.9x^{-1.671}$	12.12

Fig. 3a: Piperazine citrate equivalent anthelmintic activity of less-polar solvent fractions of *G. oppositifolius*

Among the various fractions tested PE-FC, EA-FC, DEE-FC and BUT-FC fractions showed efficient paralyzing effect within 17 min and death within 39 min at very low concentration than that of other fractions tested, where as piperazine citrate (10mg/ml) showed average time for paralysis at 22.07 min and for death at 45.5 min.

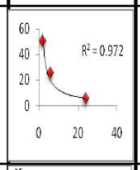
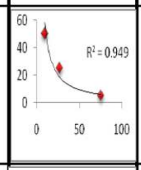
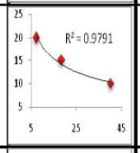
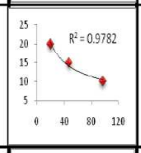
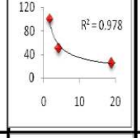
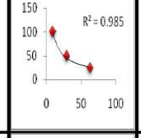
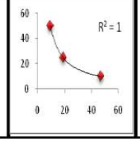
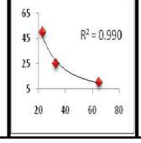
	PARALYSIS			DEATH		
SOLVENT	Graphical Representation (x axis denote Time & y axis denote Concentration)	Kinetic equation.	Conc. (mg/ml) required for performing Piperazine Citrate (10mg/ml) equiv. bioactivity	Graphical Representation (x axis denote Time & y axis denote Concentration)	Kinetic equation.	Conc. (mg/ml) required for performing Piperazine Citrate (10mg/ml) equiv. bioactivity
BUT-FC		$Y=92.237\ x^{-0.887}$	6.06		$Y=710.6\ x^{-1.112}$	10.18
MEOH-FC		$Y=45.459x^{-0.401}$	13.18		$Y=74.96x^{-0.433}$	14.35
AQU-FC		$Y=122.73\ x^{-0.555}$	22.37		$Y=470x^{-0.695}$	33.10
ACC-FC		$Y=482.95\ x^{-1.007}$	21.88		$Y=5462.3x^{-1.818}$	16.62

Fig. 3b: Piperazine citrate equivalent anthelmintic activity of more polar solvent fractions of *G. oppositifolius*

Concentration (mg/ml) of different solvent fractions required for performing piperazine citrate equivalent anthelmintic activity for paralysis and death were shown in Table 3a and 3b. The order of the paralyzing potency is, PE-FC > DEE-FC > EA-FC > BUT-FC > piperazine > MEOH-FC > CHL-FC > ACE-FC > AQU-FC > LIP-FC; whereas order of the death potency is, EA-FC > PE-FC > DEE-FC > piperazine > BUT-FC > > MEOH-FC > CHL-FC > ACE-FC > AQU-FC > LIP-FC.

Table 3: Pearson's correlation coefficient between different methods for analyzing phenolic compounds, antioxidant capacities, anthelmintic activities and extractive values

	EV	TPC	TFC	DPPH	Paralysis
TPC	-0.523^{ns}				
TFC	-0.416^{ns}	0.688*			
DPPH	0.315^{ns}	-0.603*	-0.550*		
Paralysis	0.445^{ns}	-0.816**	-0.744*	0.830**	
Death	0.558^{ns}	-0.638*	-0.630*	0.850**	0.848**

ns = non significant and *, ** = significant at P < 0.05 and 0.01 (1-tailed) respectively.

Correlations

As shown in Table 3, the parameters of anthelmintic activities (*i.e.* paralysis and death) were correlated with DPPH free radical scavenging potential with noticeable significance ($0.83 \leq r \leq 0.85$, $p < 0.01$). Also the correlation between antioxidant compounds (TPC and TFC) and anthelmintic activity were negatively high ($-0.816 \leq r \leq -0.630$, $p < 0.05$), especially between TPC and paralysis time ($r = -0.816$, $p < 0.01$). Correlations between phenolics and anthelmentic activity were negative because lesser time for paralysis and death of worms were required when polyphenols concentrations of applied extracts were augmented. Polyphenols were also negatively correlated with DPPH scavenging activity suggested that the phenolic compounds of *G. oppositifolius* leaves largely accounted for its antioxidant capacity.

Chemometric analysis

Principal Component analysis (PCA) was performed to understand the interrelationship among the measured anthelmintic activity evaluation indices, quantity of polyphenols and free-radical scavenging capacity. The analysis is based on the correlation among the variables, from which virtual axes linearly correlated to the existing variables, are generated. The data sets were standardized by the re-scaling procedure automatically to form zero mean and unit standard deviation. The original variables were linearly combined by PCA, which is responsible for the anthelmentic and antioxidant capacity of different solvent extracts. The factor loading and the variance information are shown in Table 4a and Table 4b respectively, and the results of PCA are shown in Figure 4. Two principal components, explaining the 73.4% of the total data variance, have been chosen on the basis of their eigenvalues (>1). The first component (PC1) and the second component (PC2) accounted for 43.6 and 29.8% of the total variance, respectively. The PC1 is correlated well with phenol, flavonol, paralysis and death time with component loadings of 0.842, 0.743, -0.689 and -0.704, respectively. Phenol and flavonoid contents were found to be similarly loaded whereas paralysis and death time were observed to be clustered in opposite co-ordinate with high loading on PC1, indicating that quick paralysis and death is associated with high amount of phenyl-propanoids in the extractive. Factor 2 (PC2), on

the other hand, loads moderately on the plant extract's ability to scavenge free radicals and duration of paralysis and death. PC2 clearly indicates that high radical scavenging capacity of the extracts do not necessarily translate into anthelmintic activity.

Table 4: PCA analysis table for *G. oppositifolius*

a) Component Loading

Component loading (Correlation between initial variables and principal components)		
Variable	**PC1**	**PC2**
DPPH IC_{50}	-0.611	-0.573
TPC	0.842	0.373
TFC	0.743	0.327
Paralysis	-0.689	-0.706
Death	-0.704	-0.677
EV	-0.110	-0.503

b) Component score coefficients

Component scores co-efficient (Eigenvectors)		
Variable	**PC1**	**PC2**
DPPH IC_{50}	-0.378	-0.429
TPC	0.521	0.279
TFC	0.459	0.245
Paralysis	-0.426	0.528
Death	-0.435	0.507
EV	-0.068	-0.377

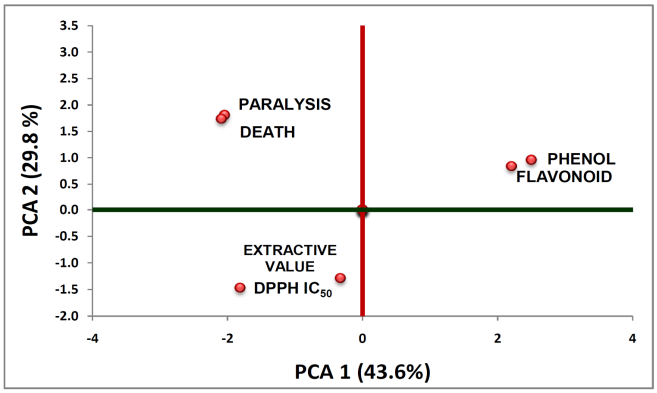


Fig. 4: Principal Component Analysis between different methods for analyzing phenolic compounds, antioxidant capacities, anthelmintic activities and extractive values.

DISCUSSION

The positive correlation between the total polyphenolic content and free radical scavenging potential had been well demonstrated in prior reports. Looking back to our results, it was observed that the PE-FC, DEE-FC, BUT-FC and EA-FC from methanol-aqueous extract of *G. oppositifolius* leaves revealed significant amount of phenyl propanoids like phenolic acids and flavonoid residues and had strong free radical scavenging activity. These findings correlate well with recent studies of *G. oppositifolius* performed by other authors. The methanolic extract of leaves of *G. oppositifolius* showed antioxidant potential, against DPPH free radicals (IC_{50} >1000µg/ml) which may be attributed to flavonoid content [27]. Similarly, the percentage of inhibition of methanolic extract of *G. oppositifolius* stem at 200 mg/ml concentration was recorded as 72.57 ± 0.153 [28], which indicates that the antioxidant activity of stem is much lesser than leaves. In our study, the diethyl ether fraction of hydro-alcoholic extract exhibited best free-radical scavenging capacity (IC_{50} = 0.176±0.002 mg/ml) among all fractions tested, which clearly indicates that diethyl ether may be the best choice for primary extraction of antioxidants.

The total phenolic content, flavonoid content and antioxidant activity of *G. oppositifolius* were determined by another author [29]. The study indicated that alcoholic extract which contained high polyphenolics, scavenged free radicals effectively. Our experimental results also supported the opinion of these authors.

Anthelmintic activity of aerial part of *G. oppositifolius* was also performed by some authors extracted separately with petroleum ether, chloroform and methanol. Among three solvents studied, only methanol extract was reported to have highest anthelmintics potential, whereas almost no activity was observed in pet ether and chloroform extract by the authors [30]. They also claimed that anthelmintics activity of this plant enhanced with increasing polarity of solvent. Interestingly our experimental observation determines that highest paralysis activity was obtained by pet ether, whereas the death potency was maximized in ethyl acetate fraction. Not only that, our results demonstrated that after fractionation, potency for paralysis and death was markedly improved particularly in different bioactive hydrophobic fractions, when compared with results of methanol extract of previous author [30]. When anthelmintic activity was considered, hydrophobic solvent may not be as effective for initial extraction as stated by previous authors but once extracted with polar solvent, the bioactive compound may be fractionated effectively through hydrophobic solvents as evidenced from our study.

The piperazine citrate produces hyper polarization and reduces excitability that leads to muscle relaxation and flaccid paralysis. These solvent fractions of *G. oppositifolius* leaves not only

demonstrated paralysis, but also cause death of worms especially at very low concentration in shorter time as compared to reference drug piperazine citrate. The total phenol and flavonoid analysis of those fractions revealed the existence of high amount of polyphenolics. Most abundant polyphenolic compounds are tannins [31] which may interact with protein excreted by nematodes in gut [32]. Tannin can also bind to glycoprotein on the cuticle of the parasite [33] and may cause death. Moreover, synthetic phenolic anthelmintics have shown to interfere with energy generation in helminth parasites muscle by uncoupling oxidative phosphorylation [13]. From our study, it may be possible that polyphenolics present in the extracts of those fractions have same anthelmintic mechanism.

The investigation of chemical compounds from natural products is fundamentally important for the development of new leads, potential for anthelmintics. Results point out that PE-FC, DEE-FC and EA-FC, obtained from hydro alcoholic extract of *G. oppositifolius* leaves are rich source of phenolics antioxidant and exhibit significant anthelmintics activity. These hydrophobic fractions may contain the bioactive compounds. However this claim demands a further quality control extraction and isolation of *G. oppositifolius* leaves. More detailed study on characterization of identified active principle could be a promising alternative to conventional anthelmintics for treatment.

ACKNOWLEDGEMENT

We are expressing our gratitude to Prof. (Dr.) A. P. Das, Taxonomy and Environmental Biology Laboratory, North Bengal University (NBU), Darjeeling, India. This study received support from UGC-DRS II. We are thankful to Management Board of Bengal School of Technology, Chinsurah, Hooghly, India for the facilities and encouragement for carrying out our research work.

REFERENCES

1. Dhar DN, Sharma RL, Bansal GC. Gastrointestinal nematodes in sheep in Kashmir. Vet Parasitol 1982; 11: 271-7.
2. El-Sokkary GH, Omar HM, Hassanein AF, Cuzzocrea S, Reiter RJ. Melatonin reduces oxidative damage and increases survival of mice infected with *Schistosoma mansoni*. Free rad Biol Med 2002; 32: 319-32.
3. El-Shenawy NS, Soliman MF, Reyad SI. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. Rev Inst Med Trop Sao Paulo 2008; 50: 29-36
4. Bundy DA, Immunoepidemiology of intestinal helminthic infection 1: The global burden of intestinal nematode disease. Trans R Soc Trop Med Hyg 1994:88 Suppl 3: 259-61.
5. Tagbota S, Townson S. Antiparasitic properties of medicinal and other naturally occurring products. Adv Parasitol 2001; 50: 199-295.
6. Waller PJ. Anthelminthic resistance. Vet Parasitol 1997; 72: 391-405
7. Singh D, Swarnakar CP, Khan FA. Anthelmintic resistance in gastrointestinal nematodes of livestock in India. J Vet Parasitol 2002; 16: 115-30
8. Geert S, Dorny P. Anthelmintic resistance in helminthes of animals and man in the tropics. Bull Séances Acad R Sci Outre Mer 1995; 3: 401–23.
9. Coles GC. Nematode control practices and anthelmintic resistance on British sheep farms. Vet Rec 1997; 141: 91–3.
10. Satyavati GV, Raina MK, Sharma M. Medicinal Plants of India. Vol I. New Delhi: Indian Council of Med Res; 1976. p. 201– 6.
11. Nadkarni AK. Dr K. M. Nadkarni's Indian Materia Medica, with Ayurvedic, Unani-tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendicces & indexes. 3rd ed. Vol 1. Bombay, India: Popular Prakashan; 1954.p. 181
12. Akhtar MS, Iqbal Z, Khan MN, Lateef M. Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. Small Rumin Res 2000; 38: 99–107.
13. Niezen JH, Waghorn GC, Charleston WAG, Waghorn GC. Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*), which contains condensed tannins. J Agri Sci 1995; 125: 281–9.
14. Wadekar RR, Wani NS, Bagul UB, Bagul SD, Bedmutha RK. Phytochemical investigation and screening of *in vitro* anthelmintic activity of *Plectranthus amboinicus* leaves extracts. Int J Pharmacogn Phytochem Res 2011; 3: 35-8
15. Shetgiri PP, D'Mello PM. Antioxidant activity of flavonoids: A comparative study. Ind Drugs 2003; 40: 567-9
16. Jain N, Goyal S, Ramawat KG. Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare in rajasthan. Int J Pharm Pharm Sci, 2011; 3 Suppl 3: 248-53.
17. Diallo D, Marston A, Terreaux C, Touré Y, Paulsen BS, Hostettmann K. Screening of Malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. Phytother Res 2001; 15: 401-6
18. Vigar Z. Atlas of Medical Parasitology. 2nd ed. Singapur: P G Publishing House; 1984. p. 242.
19. Dash GK, Suresh P, Sahu SK, Kar DM, Ganpaty S, Panda SB. Evaluation of *Evolvulus alsinoides* Linn. for anthelmintic and antimicrobial activities. J Nat Rem 2002; 2: 182-5.
20. Shivkumar YM, Kumar VL. Anthelmintic activity of latex of *Calotropis procera*. Pharma Biol 2003; 41: 263-5
21. Chandler SF, Dodds JH. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum*. Plant Cell Rep 1983; 2: 105–8
22. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009; 14: 2167-80
23. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniae* flora. J Ethnopharmacol 2002; 79 Suppl 3: 379-81
24. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm Biol 2001; 39: 217-20.
25. Kaushik RK, Katiyar JC, Sen AB. Studies on the mode of action of anthelmintics with *Ascardia galli* as a test parasite. Indian J Med Res 1974; 62: 1367-75
26. Nayak BS, Jena PK, Sahu NP, Nayak UK, Patro KB. Comparative study of anthelmintic activity between aqueous and ethanolic extract of *Solanum surattense* Linn. Int J Pharm Pharm Sci 2009; 1 Suppl 1: 103-7
27. Hoque N, Imam MZ, Akter S, Mazumder MEH, Hasan SMR, Ahmed J, *et al*. Antioxidant and antihyperglycemic activities of methanolic extract of *Glinus oppositifolius* leaves. J App Pharm Sci 2011; 1: 50-3
28. Behera GM, Kurnool AN, Baidya M, Satish Kumar BN, Bilal S. Anti-hyperglyceamic, anti-hyperlipidemic and antioxidant activity of the stem of *Glinus oppositifolius* (L.) Aug. DC. Int J Ph Sci 2011; 3: 1080-90
29. Ashokkumar K, Umamaheswari M, Sivashanmugam AT, Subhadradevi V, Subhashini N, Ravi TK. Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpet weed) using different *in vitro* assay systems. Pharmaceut Bio 2009; 47: 474-82
30. Pattanayak S , Nayak SS, Dinda SC, Panda DP, Kolhe D. Antimicrobial and anthelmintic potential of *Glinus oppositifolius* (Linn) Family: Molluginaceae. Pharmacologyonline 2011; 1: 165-9
31. Martin RJ. γ- Aminobutyric acid and Piperazine activated single channel current from *Ascaris suum* body muscle. Br J Pharmacol 1985; 84: 445-61.
32. McKellar QA, Duncan JL, Armour J, Lindsay FEF, McWilliam P. Further studies on the response to transplanted adult *Ostertagia ostertagi* in calves. Res Vet Sci 1987; 42: 29–34
33. Martin RJ. Mode of action of anthelmintic drugs. Vet J 1997; 154: 11-34.