International Journal of Biomedical and Pharmaceutical Sciences ©2011 Global Science Books

Anthelmintic, Free-Radical Scavenging Property and Potent Antibacterial Activity of Flavonoid Fraction Isolated from the Whole Plant of *Oldenlandia corymbosa* L.

Himangshu Sekhar Maji[1*] • Sushomasri Maji[1] • Kausik Bhar[1] • Jaishree Chandra[1] • Manik Baral[1] • Pranabesh Chakraborty[2] • Sujata Ghosh Dastidar[3]

[1] Gupta College of Technological Sciences, Asansol, West Bengal - 713301, India
[2] SEDCO, Chandannagor, Hooghly, India
[3] Herbicure Healthcare Bioherbal Research Foundation, Pailan, Kolkata - 700104, India

Corresponding author: * **hs_maji@yahoo.co.in**

ABSTRACT

The flavonoid fraction isolated from the ethanolic extract of the whole plant of *Oldenlandia corymbosa* L. (Family: Rubiaceae) exhibited distinct antibacterial activity when tested against 15 bacterial strains of different genera. Minimum inhibitory concentration (MIC) of the flavonoid fraction was determined following NCCLS (2003) guidelines using the agar dilution method. *Micrococcus lutea* and *Salmonella typhi* 7652 were inhibited at the lowest concentration (25 µg/ml), while other bacteria were inhibited at < 200 µg/ml. The ethanolic extract when tested for possible anthelmintic activity to determine the paralyzing and death time on the aquarium worm, *Tubifex tubifex*. The ethanolic extract showed anthelmintic property comparable to the reference drug piperazine citrate at 80 mg/ml. Various phytochemical tests performed on the ethanolic extracts suggest that the plant contains reducing sugars, amino acids, steroids, flavonoids, alkaloids and glycosides. The active phytocomponent (flavonoid) was isolated by TLC, column chromatography and characterized using various analytical techniques like CHN analysis, UV-Vis spectroscopy, FT-IR, ^{1}H NMR and mass spectral analysis. The presence of flavonoids in the ethanolic fraction prompted us to search for its free radical scavenging property. The IC_{50} value by DPPH radical scavenging activity of the crude ethanolic fraction containing the flavonoid part was 560 µg/ml. Though this value was not comparable to the reference compound BHT but it indicated the urge to examine the isolated flavonoids *in vivo* for their free radical scavenging activity.

Keywords: bacteriostatic, benign, inhibitory, phytocomponent, radical
Abbreviations: **BHT**, butylated hydroxy toluene; **DCA**, desoxycholate citrate agar; **DPPH**, 2, 2-diphenyl-1-picrylhydrazyl; **MHA**, Muller-Hinton agar; **MHB**, Muller-Hinton broth **MIC**, minimum inhibitory concentration; **NCCLS**, National Committee for Clinical Laboratory Standards; **PW**, peptone water; **TLC**, thin layer chromatography

INTRODUCTION

For both developed and developing countries, recognition and development of herbal medicine offer treatment methods that are more environmentally benign, since they tend to be less toxic, produce fewer unanticipated side effects and apparently do not trigger anthelmintic chemo resistance (Sujon *et al*. 2008). Herbal medicine represents one of the most important fields of traditional medicine in India. A number of traditional medicinal plants have been used in folk medicine to treat a wide range of human physical ailments. The traditional medicines all over the world are nowadays reevaluated by an extensive activity of research on different plant species and their therapeutic principles (WHO 2002). Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. Plants are known to provide cures for various human illnesses and are a rich source of phytoconstituents having diversified pharmacological properties.

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari 2001). The most common reactive oxygen species (ROS) include superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO^-) radicals and reactive hydroxyl (OH^-) radicals. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a number of diseases (Joyee 1987; Richards and Sharma 1991; Niwa 1991). Antioxidant therapy has gained immense importance in treating those diseases

Fig. 1 ***Oldenlandia corymbosa*** **with flowers and seeds.**

(Buyukokuroglu *et al*. 2001). Flavonoids and phenolic compounds, widely distributed in plants, have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory and anticarcinogenic, etc. (Miller 1996; Adeolu *et al*. 2008). The intake of natural antioxidants has been associated with reduced risk of cancer, cardiovascular diseases, diabetes and other diseases associated with ageing (Mclarty 1997; Yang *et al*. 2001).

The discovery of antibiotics and antibacterial chemotherapeutics acted like a magic bullets against microbial infections in the beginning. However, the massive and indiscriminate use of antibiotics caused the earlier euphoria to evaporate, replacing it with the occurrence of drug-resistant

Received: 6 February, 2010. Accepted: 29 June, 2011.

Original Research Paper

bacteria. Studies in this line have disclosed notable antimicrobial action in drugs belonging to different pharmacological classes such as antihistamines like trimeprazine (Dastidar *et al*. 1997), tranquillizers like promazine (Chakraborty *et al*. 1977), antihypertensives like amlodipine (Dutta *et al.* 2009), lacidipine (Dasgupta *et al*. 2007), antipsychotics like phenothiazines (Amaral *et al*. 2007), and the anti-inflammatory agent diclofenac (Dutta *et al.* 2007). Such drugs, having antimicrobial activity in addition to their predesignated pharmacological action, have been grouped together under the banner of "non-antibiotics" (Kristiansen 1992). Pursuing this line of thought scientists from divergent fields are investigating plants with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles (Cowan 1999).

Oldenlandia corymbosa (F: Rubiaceae) (Kirtikar and Basu 2001) (**Fig. 1**), which is a common weed found in both dry and wet lands throughout India, has been used in traditional medicine of India and China (Liang *et al.* 2008) to treat various hepatic disorders and as an immunity enhancer. Although *O. corymbosa* (syn. *Hedyotis corymbosa*) is described as a problematic weed in the literature of weed science but for traditional healers this wasteland herb is a herb of promising medicinal properties and uses (Chikoye *et al*. 2007; Kamal-Uddin *et al*. 2009; Brecke *et al*. 2010). It is an annual herb with numerous stem; leaves subsessile, linear or linear-lanceolate, margins recurved and scabrous, stipules with bristles; flowers on filliform pedicels usually 2-3 cm long, lobes acute; fruit capsule, globose or pyriform; seeds pale brown, angular, germinate at high temperature (Corbineau *et al*. 1982; Corbineau *et al*. 1985); flowering time October to December. The whole plant of *Oldenlandia* is used as medicine (Noiarsa *et al*. 2008). Sanskrit authors consider it a cooling medicine of importance in the treatment of fever (remittent fever with gastric irritability) and nervous depressions; a decoction of the whole plant is said to be a good febrifuge and is used in chronic malaria (Mishra *et al.* 2009); it is used in liver complaints (Sadasivan *et al*. 2006). In the Konkon region of India, juice of plant is applied to palms and solves when they burn from fever (Kirtikar and Basu 2001). Juice is given internally with a little milk and sugar in the burning at the stomach pit and to cure heart disease.

Therefore, the objective of the present study is to find the phytochemically important constituents present and evaluate antibacterial, anthelmintic and free radical scavenging property of important constituent present in *O. corymbosa.* The present study helps to establish effective, available and low cost medicines of plant origin.

MATERIALS AND METHODS

Plant material

Fresh plants were collected from the Haripal of Hooghly district and medicinal plants garden, Gupta College of Technological Sciences, Asansol, India. The collected plants were identified and authenticated from the Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen (Specimen No. CNH/I-I/2008/Tech.II/) was deposited at the Central National Herbarium, Shibpur, India for future reference.

Animals

Tubifex tubifex were collected from local market of Asansol, India. Because of easy availability and due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings, *T. tubifex* have been used widely for the initial evaluation of anthelmintic compounds.

Chemicals

All chemicals and solvents used were of analytical grade available commercially. DPPH (2, 2-diphenyl-1-picryl hydrazyl) was procured from ICN Biomedicals Inc. Ohio, US; BHT (butylated hydroxy toluene/2,6 di-tert-butyl-*p*-cresol) was purchased from Loba Chemie Pvt. Ltd., India; piperazine citrate was procured from SRL, India.

Media

Liquid media used for this study were peptone water [PW; Oxoid brand bacteriological peptone 1% (w/v) plus Analar NaCl 0.5% (w/v)], nutrient broth (NB; Oxoid), Muller-Hinton broth (MHB; Difco). Solid media were desoxycholate citrate agar (DCA, Oxoid) and peptone agar (PA), nutrient agar (NA) and Muller-Hinton agar (MHA), obtained by solidifying the liquid media with 1.2% (w/v) agar (Oxoid No. 3). The pH was maintained at 7.2-7.4 for all the media. For isolation, identification and maintenance NA and PW was used for Gram-positive bacteria, PA and DCA were used for the rest, while MHA and MHB were used for evaluating antimicrobial activity of the isolated extract.

Preparation of extract

Whole *O. corymbosa* plants were shade dried at room temperature after collection and was powdered and passed through 60-120 mesh prior to extraction. The powder (approximately 85 g) was extracted with petroleum ether, chloroform and ethanol separately by continuous hot percolation in a Soxhlet apparatus for 8-10 cycles each. Solvents were recovered by distillation and ethanol extracts were concentrated in reduced pressure below 40°C in a rotary flash evaporator (ROLEX) to obtain a crude ethanol extract (14.2%, w/w). This was then stored at 4°C until further use.

In vitro anthelmintic activity

The anthelmintic assay was carried as per the method of Ajaiyeoba *et al.* (2001) with minor modifications. The assay was performed on an adult Indian earthworm, *Tubifex tubifex* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Vidyarthi 1967; Thorn *et al.* 1977). All the test solution and standard solutions were prepared freshly before commencement of the experiment. 20 ml of formulations containing three different concentrations of ethanolic extract (10, 25, 50 and 80 mg/ml in double distilled water) were prepared in a 500 ml beaker and six young *Tubifex* worms collected from the local market and maintained at standard laboratory conditions were placed into it. Paralysis occurred when no movement of any sort could be observed except when the worms were shaken vigorously. Death of worms occurred when they neither moved when shaken vigorously or when dipped in warm water (50°C). Piperazine citrate (10 mg/ml) was used as the reference standard while distilled water served as the control (Mali *et al*. 2004, 2005). Animal experiments were carried out following guidelines of institutional animal ethics committee.

DPPH radical scavenging assay

The effect of the ethanolic extract on DPPH radical was determined using the method of Liyana-Pathiranan (2005). A 0.135 mM DPPH solution and various concentrations (0.02-0.1 mg/ml) of extracts were prepared in methanol. 1.0 ml of DPPH solution was mixed with 1.0 ml of various test solutions and incubated in the dark at 25°C for 20 min after vortexing thoroughly. The absorbance was read at 517 nm. A control reaction was carried out without the extract. BHT was used as reference. A linear graph of concentration vs. percentage inhibition was prepared and IC_{50} values were calculated. The % inhibition was calculated by the following formula (Jain *et al*. 2008):

$$\% \text{ Inhibition} = (A_0 - A_t)/A_0 \times 100$$

where A_0 was the absorbance of the control and A_t was the absorbance in the presence of ethanolic extract of *O. corymbosa*.

Table 1 Effect of ethanolic extract of *Oldenlandia corymbosa* on death time (D) and paralyzing time (P) *Tubifex tubifex*.

Category	Concentration (mg/ml)	Paralyzing (P) time minutes	Death (D) time minutes
Water (Control)	-	-	-
Extract	10	65.33 ± 0.52	94.83 ± 1.3
	25	54.67 ± 0.52	69.17 ± 1.6
	50	23.33 ± 1.03	44.67 ± 0.51
	80	18.33 ± 1.5	31.67 ± 1.75
Piperazine citrate	10	21.17 ± 0.75	45.33 ± 1.03

Values are mean ± SD (n=6)

Table 2 Effect (% inhibition) of ethanolic extract of whole plant of *Oldenlandia corymbosa* on DPPH radical scavenging assay.

Concentration of extract (mg/ml)	% Inhibition	Concentration of BHT (µg/ml)	% Inhibition
0	0	0	0
0.2	38.6 ± 0.4	10	22.25 ± 0.26
0.4	49.57 ± 0.31	20	38.75 ± 0.26
0.6	57.36 ± 0.21	25	55.6 ± 0.10
0.8	64.27 ± 0.21	30	72.4 ± 0.1
1.0	69.4 ± 0.17	50	94.47 ± 0.03
IC_{50}	0.56 mg/ml	IC_{50}	23.9 µg/ml

Values are mean ± SD (n=3), IC_{50} = 50% inhibition concentration

Determination of MIC of flavonoid obtained from ethanolic extract of *O. corymbosa*

The minimum inhibitory concentration (MIC) of the extract with respect to different test bacteria was accurately determined by agar dilution methods. For agar dilution, 1 ml of the flavonoid fraction of the extract was added at concentrations of 0 (control), 10, 25, 50, 100, 200 and 400 µg/ml in 9 ml molten MHA and poured into sterile 40 mm diameter Petri dishes (Koneman 1997). The organisms were grown in MHB and the overnight culture was spot inoculated onto MHA plates such that each inoculum contained 2 × 10^5 CFUs (colony-forming units). The plates were incubated at 37°C, examined after 24 h for any growth and incubated for a further 72 h for satisfactory growth. Since one MHA medium containing the extract can be used for inoculation of a large number of bacteria at a time, the result of this method is presented in tabular form (**Table 4**). The lowest concentration of extract in a plate that failed to show any visible growth was considered as its MIC.

Statistical analysis

The MIC determination was performed in duplicate for each organism. For anthelmintic and antioxidant activity tests were performed in triplicate. Experimental results are mean ±SD for three parallel measurements. IC_{50} value was graphically determined and means, standard deviation and correlation were computed for both the experiments by using Microsoft excel.

RESULTS

In vitro anthelmintic activity of ethanol extract of the whole plant of *O. corymbosa*

The ethanolic extract of *O. corymbosa* exhibited significant anthelmintic activity at higher concentrations (**Table 1; Fig. 2**). The extract showed anthelmintic activity in a dose-dependent manner giving shortest time of paralysis (P) and death (D) at 80 mg/ml. The alcoholic extract of *O. corymbosa* caused paralysis in 18 min and death in 32 min (**Fig. 2**), while the reference drug piperazine citrate showed P and D in 21 and 45 min, respectively against the earthworm *Tubifex tubifex*.

Hydroxyl radical scavenging activity

The ethanolic extract of the whole plant of *O. corymbosa* was evaluated for antioxidant activity by using the DPPH free radical scavenging method. The extract has promising free radical scavenging power (**Table 2; Figs. 3, 4**). The IC_{50} value was 560 µg/ml.

Phytochemical evaluation and isolation of flavonoids

Various phytochemical tests done on petroleum ether, chloroform and ethanol extracts suggest that the plant contains reducing sugars, amino acids, steroids, flavonoids, alkaloids and glycosides. Flavonoids were isolated from the ethanol fraction chromatographically by TLC and column chromatography with butanol, acetic acid and water (8: 1: 1) and was subsequently characterized using different analytical

Table 3 Analytical profile of the ethanolic extract of the whole plant of *Oldenlandia corymbosa.*

Methods adopted	Results
Phytochemical screening	Flavonoid, glycoside
Ash value	14%
TLC (silica gel) (BuOH, CH_3COOH, H_2O 8:1:1)	Detection UV, R_f 0.69
CHN analysis	C 32.15%, H 6.32%, N 1.64%
UV (MeOH)	λ_{max} 247 nm
FT-IR in KBr	3420.85 (O-H str.), 2922 & 2852.26 (C-H str.), 1735.98 (C=O str.), 1647.98 (N-H def.), 1560, 1261.39 (C-O-C str), 1075.84 cm^{-1}
^{1}H NMR	0.833 δ 1(H), 1.208 δ 1(H), 1.476 δ, 3.177 δ 18(H), 3.7 δ H(RCOOH), 2.6 δ H(C-CH_3)
Mass spectra (ESI)	m/z: 632.89, 426.92, 300.99, 258.99 (base peak), 230.99, 202.95

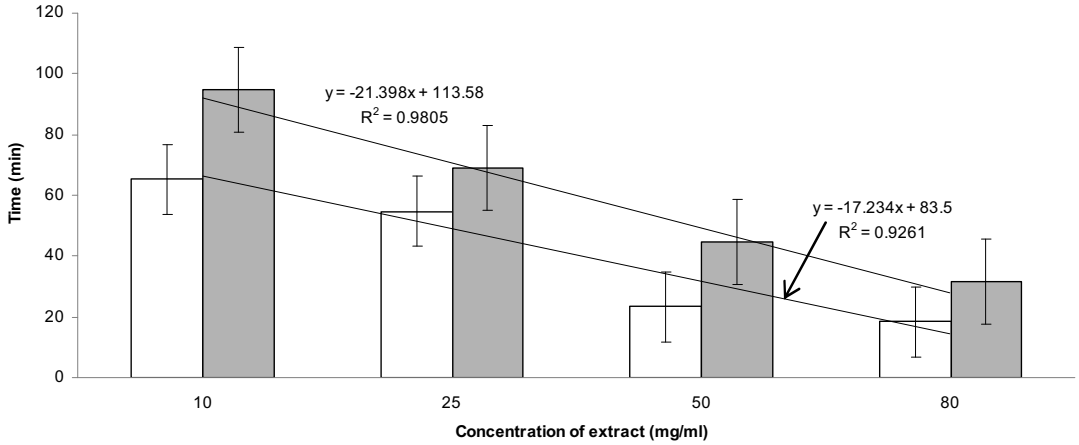

Fig. 2 Effect of ethanolic extract of *Oldenlandia corymbosa* on death time (D) and paralyzing time (P) *Tubifex tubifex*. □ Paralyzing time (P); ■ Death time (D).

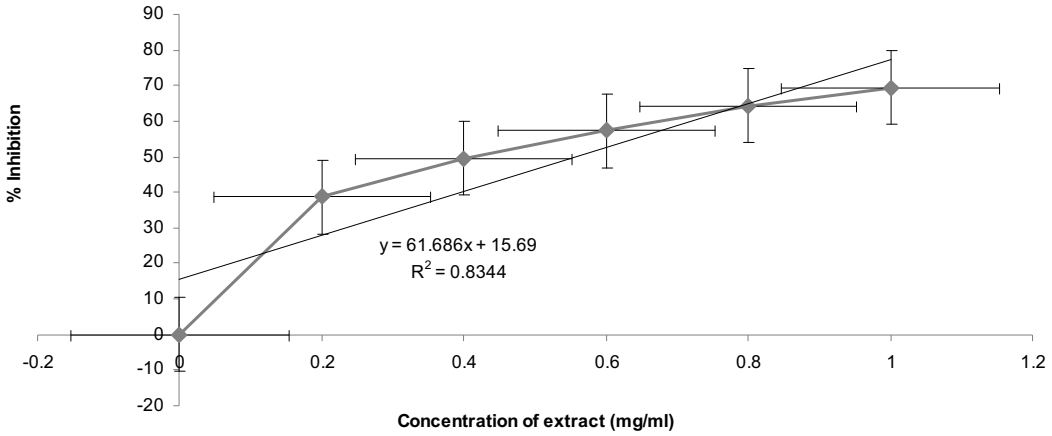

Fig. 3 Effect (% inhibition) of ethanolic extract on DPPH radical scavenging assay.

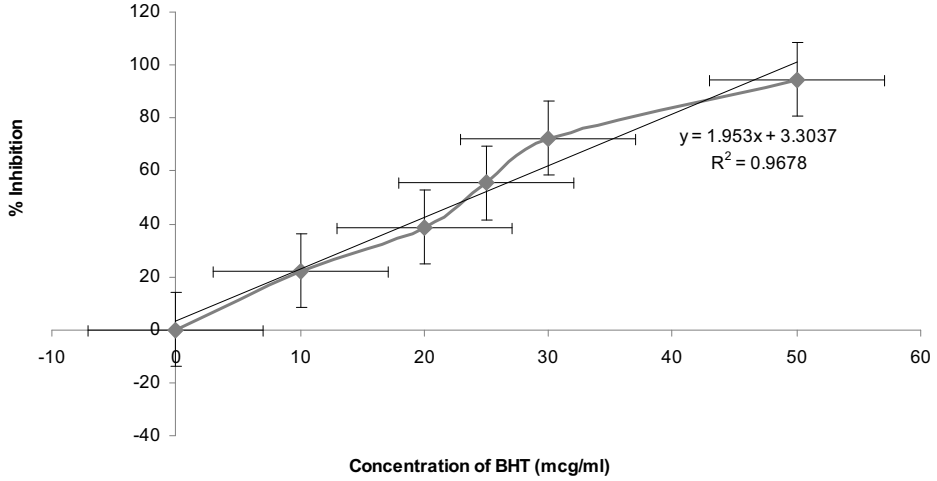

Fig. 4 Effect (% inhibition) of BHT on DPPH radical scavenging assay.

techniques like UV-Vis spectroscopy (UV-1, Thermo Spectronic), FT-IR (RX-1, Perkin-Elmer), ^{1}H NMR (DPX-300, BRUKER) and Mass (Qtof Micro YA 263, WATERS) spectral analysis and CHN analysis (2400, Perkin Elmer). The results of various spectroscopic and chromatographic data in **Table 3** further confirm the presence of flavonoids as found in preliminary phytochemical screening.

In vitro antimicrobial activity of *O. corymbosa*

Screening the ethanolic extract of *O. corymbosa* against 15 known sensitive bacteria belonging to both Gram-positive and -negative genera showed a powerful antimicrobial action against most of the tested bacteria (**Table 4**).

DISCUSSION

O. corymbosa is a common weed found in wastelands throughout India. It has use in traditional medicine of India and China to treat various hepatic disorders. *O. corymbosa* is rich in ascorbic acid when compared with some common garden fruits and vegetables (HAO and PAO 2006).

The ethanolic extract of *O. corymbosa* not only demonstrated paralysis, but also caused death of worms especially at a higher concentration, i.e., 80 mg/ml (**Table 1; Fig. 2**), comparable to the reference drug piperazine citrate. Phytochemical analysis of the crude extracts revealed the presence of alkaloids as one chemical constituent. Chemically, alkaloids are polyphenolic compounds. Some synthetic

Table 4 Effect of *Oldenlandia corymbosa* on different bacteria.

Bacterial strains tested	Growth in nutrient agar containing different concentrations of extract (µg/ml)						
	0**	10	25	50	100	200	400
B. subtilis NCTC8241	+	+	+	+	-	-	-
E. coli K_{12} Row	+	+	+	+	-	-	-
K. pneumoniae 14	+	+	+	+	+	-	-
Lactobacillus sporogens	+	+	+	+	-	-	-
Micrococcus lutea ATCC 9341	+	+	-	-	-	-	-
Ps. aeruginosa APC 1	+	+	+	+	+	-	-
S. aureus ATCC29157	+	+	-	-	-	-	-
S. aureus NCTC6571	+	+	-	-	-	-	-
S. typhi 7652	+	+	-	-	-	-	-
S. typhimurium NCTC74	+	+	+	-	-	-	-
Sh. boydii 8NCTC254/66	+	+	-	-	-	-	-
Sh. sonnei 1NCTC9774	+	+	-	-	-	-	-
St. faecalis S_1	+	+	+	+	+	-	-
V. cholerae 865	+	+	-	-	-	-	-
V. cholerae ATCC14033	+	+	-	-	-	-	-

** Control plate without extract; +, growth; -, no growth

phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin 1997). It is possible that alkaloids contained in the extracts of *O. corymbosa* produced similar effects.

The potential scavenging abilities of polyphenols might be due to active oxygen donor ability of hydroxyl substitution (Bose *et al*. 2008). Similarly, high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging by specific functional groups (Korycka-Dahl and Richardson 1978). Our *in vitro* antioxidant study (**Table 2; Figs. 3, 4**) provides immense scientific support to use this plant effectively against free radical-mediated diseases.

Since the preliminary screenings of the ethanolic extract have shown the presence of important phytoconstituents (glycosides and flavonoids), this fraction was chosen to evaluate of pharmacological and antibacterial potential.

Bacterial cells multiply repeatedly and cause infections in various organs of the human body. For such rapid replication, the organisms must synthesize or take up many types of biomolecules. Therefore, effective antimicrobial agents must interfere with specific processes that are essential for growth and/or division. Antimicrobial agents may be either cidal (killing the invading pathogens), or static (inhibiting growth). Bactericidal agents are more effective, but bacteriostatic agents can be extremely beneficial, since they permit the normal defenses of the host to destroy the microorganisms (Gale *et al*. 1981; Waxman and Strominger 1982). An extensive review of the literature (Dastidar *et al*. 2001, 2004; Mishra *et al*. 2009) indicated that antimicrobial activity of different non-antibiotics might be due to the presence of aromatic rings present in flavonoids. From this postulate and from phytochemical screening of the ethanolic extract an attempt was made to find the possible antibacterial activity of *O. corymbosa*. The ethanolic extract of the flavonoidal fraction shows significant antibacterial activity (**Table 4**) which needs to be further tested *in vivo*.

All the results were found to be significant statistically and thus it can be concluded that *O. corymbosa* is a potential plant with strong antibacterial and anthelmintic properties and with distinguished free radical scavenging property. The plant needs to be further evaluated in combination with other plants of the same plant family to establish this common weed as a pharmacologically potential herb.

ACKNOWLEDGEMENTS

The corresponding author is thankful to Mr. M. S. Mondal, Joint Director, Botanical Survey of India, Howrah, India for identification of the plant.

REFERENCES

Adeolu AA, Jimoh FO, Afolayan AJ, Masika PJ (2008) Antioxidant activities and phenolic contents of the metanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera. BMC Complementary and Alternative Medicine* **8 (54)**, 1-7

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001) *In vitro* anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract. *Pharmaceutical Biology* **39**, 217-20

Amaral L, Martins M, Viveiros M (2007) Phenothiazines as anti-multi-drug resistant tubercular agents. *Infectious Disorders-Drug Targets* 7, 257-265

Bose S, Bose A, Maji S, Chakraborty P (2008) *In vitro* antioxidant property of leaf extract of *Ixora coccinea* L. *International Journal of Biomedical and Pharmaceutical Sciences* **2 (2)**, 84-87

Brecke BJ, Daniel OS, Unruh JB (2010) Timing of oxadiazon and quinclorac application on newly sprigged turfgrass species. *Weed Technology* **24 (1)**, 28-32

Buyukokuroglu ME, Gulcin I, Oktay M, Kufrevioglu OI (2001) *In vitro* antioxidant properties of dantrolene sodium. *Pharmacological Research* **44**, 491-495

Chakraborty P, Dash SK, Dastidar SG (1977) Antimicrobial activity of promazine hydrochloride. *Indian Journal of Experimental Biology* **15**, 324-326

Chikoye D, Udensi EU, Fontem LA, Friday E (2007) Rimsulfuron for post-emergence weed control in corn in humid tropical environment of Nigeria. *Weed Technology* **21 (4)**, 977-981

Corbineae F, Daniel C (1982) Effect of the intensity and duration of light at various temperatures on the germination of *Oldenlandia corymbosa* L. seeds. *Plant Physiology* **70**, 1518-1520

Corbineae F, Daniel C (1985) Effect of the temperature, oxygen and gibberellic acid on the development of photosensitivity in *Oldenlandia corymbosa* L. seeds during their incubation in darkness. *Plant Physiology* **79**, 411-414

Cowan MM (1999) Plant products as antimicrobial agents. *Clinical Microbiology Review* **12**, 564-582

Dasgupta A, Jeyaseeli L, Dutta NK, Mazumdar K, Karak P, Dastidar SG, Motohashi N, Shirataki Y (2007) Studies on the antimicrobial potential of the cardiovascular drug lacidipine. *In Vivo* **21**, 847-850

Dastidar SG, Jairaj J, Mookerjee M, Chakrabarty AN (1997) Studies on antimicrobial effect of the antihistaminic phenothiazine trimeprazine tartarate. *Acta Microbiologica et Immunologica Hungarica* **44**, 241-247

Dastidar SG, Mahapatra SK, Ganguly K, Chakraborty AN, Shirataki Y, Motohashi N (2001) Antimicrobial activity of prenylflavonones. *In Vivo* **15**, 519-524

Dastidar SG, Manna A, Asok Kumar A, Mazumdar K, Dutta NK, Chakraborty AN, Motohashi N, Shirataki Y (2004) Studies on the antibacterial potentiality of isoflavones. *International Journal of Antimicrobial Agents* **23**, 99-102

Dutta NK, Mazumdar K, Dastidar SG, Park J (2007) Activity of diclofenac used alone and in combination with streptomycin against *Mycobacterium tuberculosis* in mice. *International Journal of Antimicrobial Agents* **30 (4)**, 336-340

Dutta NK, Mazumdar K, Dasgupta A, Dastidar SG (2009) *In vitro* and *in vivo* efficacies of amlodipine against *Listera monocytogenes. European Journal of Clinical Microbiology and Infectious Diseases* **28 (7)**, 849-853

Gale EF, Cundliff E, Reynolds PE (1981) *The Molecular basis of Antimicrobial Action* (2nd Edn), John Wiley & Sons, New York, pp 402-547

Jain PK, Agrawal RK (2008) Antioxidant and Free radical scavenging properties of developed mono- and polyherbal formulations. *Asian Journal of Experimental Sciences* **22 (3)**, 213-220

Joyee DA (1987) Oxygen radicals in disease. *Adverse Drug Reaction Bulletin* **127**, 476-479

Kamal-Uddin M, Juraimi AS, Begam M, Ismail MR, Rahim AA, Othman R (2009) Floristic composition of weed community in turf grass area of west peninsular Malaysia. *International Journal of Agriculture and Biology* **11**, 13-20

Kirtikar KR, Basu BD (2001) *Indian Medicinal Plants with Illustrations* (2nd Edn, Vol 6) Oriental Enterprise, Dehradun, Uttaranchal, India, pp 1748-1750

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. (1997) *Colour Atlas and Textbook of Microbiology* (6th Edn), Lippincott, USA, pp 921-938

Korycka-Dahl M, Richardson M (1978) Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids. *Journal of Dairy Science* **61**, 400-407

Kristiansen JE, Thomsen VF, Hvidberg EF (1992) The antimicrobial activity of non-antibiotics. *Acta Pathlogica Microbiologica et Immunologica Scandinavica* **100 (Suppl. 30)**, 7-19

Liana-Pathiranan CM, Shahidi F (2005) Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agriculture and Food Chemistry* **53**, 2433-2440

Liang Z, He M, Fong W, Jiang Z, Zhang Z (2008) A comparable, chemical and pharmacological analysis of the traditional Chinese medicinal herbs *Oldenlandia diffusa* and *O. corymbosa* and a new valuation of their biological potential. *Phytomedicine* **15 (4)**, 259-267

Mali RG, Hundiwale JC, Sonawane RS, Patil RN, Hatapakki BC (2004) Evaluation of *Capparis decidua* for anthelmintic and antimicrobial activities. *Indian Journal of Natural Product* **20**, 10-13

Mali RG, Shailaja Mahajan, Patil KS (2005) Anthelmintic activity of root bark of *Capparis spinosa. Indian Journal of Natural Products* **21**, 50-51

Martin RJ (1997) Mode of action of anthelmintic drugs. *The Veterinary Journal* **154**, 11-34

McLarty JW (1997) Antioxidant and cancer: The epidemiological evidence. In: Garewal HS (Ed) *Antioxidants and Disease Prevention*, CRC Press, Boca Raton, FL, pp 45-66

Miller AL (1996) Antioxidant flavonoids: Structure, function and clinical usage. *Alternative Medicine Review* **1**, 103-111

Mishra K, Das A, Swain BK, Dey N (2009) Anti-malarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin. *Malaria Journal* **1**, 8-26

Mishra US, Chakraborty P, Dasgupta A, Dastidar SG, Martins M, Amaral L (2009) Potent bactericidal action of a flavonoid fraction isolated from the stem bark of *Butea frondosa*. *In Vivo* **23**, 29-32

Okeri HA, Alonge PO (2006) Determination of the ascorbic acid content of two medicinal plants in Nigeria. *Pakistan Journal of Pharmaceutical Sciences* **19 (1)**, 44-48

National Committee for Clinical Laboratory Standards (2003) Performance standards for antimicrobial disc susceptibility tests; Approved Standards, M2-A8, NCCLS, Wayne, PA

Niwa Y (1991) Effect of Maharishi 4 and Maharishi 5 on inflammatory mediators with special reference to their free radical scavenging effect. *Journal of*

Clinical Practice **1**, 23-27

Noiarsa P, Ruchirawat S, Otsuka H, Tripetch K (2008) Chemical constituents of *Oldenlandia corymbosa* L. of Thai origin. *Journal of Natural Medicine* **62 (2)**, 249-250

Richards RT, Sharma HM (1991) Free radicals in health and disease. *Indian Journal of Clinical Practice* **2**, 15-26

Sadasivan S, Latha PG, Sasikumar JM, Rajashekaran S, Shyamal S, Shine VJ (2006) Hepatoprotective studies on *Hedyotis corymbosa* (L.) Lam. *Journal of Ethnopharmacology* **106 (2)**, 245-249

Sujon MA, Mostofa M, Jahan MS, Das AR, Rob S (2008) Studies on medicinal plants against gastrointestinal nematodes of goats. *Bangladesh Journal of Natural Medicine* **6 (2)**, 179-183

Thorn GW, Adams RD, Braunwald E, Isselbacher KJ, Petersdorf RG (1977) *Harrison's Principles of Internal Medicine*, McGraw Hill Co., New York, 1088 pp

Tiwari A (2001) Imbalance in antioxidant defence and human diseases: multiple approach of natural antioxidant therapy. *Current Science* **81**, 1179-1187

Vidyarthi RD (1967) *A Text Book of Zoology* (1th Edn), S. Chand and Co, New Delhi, 329 pp

Waxman DJ, Strominger JL (1982) Beta-lactam antibiotics: Biochemical modes of action. In: Morin RB, Gorman M (Eds) *Chemistry and Biology of Beta Lactam Antibiotics*, Academic Press, San Diego, 210 pp

World Health Organization (2002) WHO traditional medicine strategy 2002-2005. WHO, Geneva, Switzerland, pp 1-48

Yang CS, Landau JM, Huang MT, Newmark HL (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Review of Nutrition* **21**, 381-406