



BCSIR

Available online at www.banglajol.info
Bangladesh J. Sci. Ind. Res. **48(1)**, 43-50, 2013

**BANGLADESH JOURNAL
OF SCIENTIFIC AND
INDUSTRIAL RESEARCH**

E-mail: bjisir07@gmail.com

Phytochemical and biological activities of ethanolic extract of *C. hirsuta* leaves

M. A. Rahman^{1*}, M. A. Rahman² and N. U. Ahmed²

¹*Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh*

²*Bangladesh Council of Scientific and Industrial Research, Rajshahi*

Abstract

This study investigated the phytochemical and biological activities like antioxidant, antibacterial and cytotoxic effects of *C. hirsuta* (Synonym- *Senna hirsuta*) leaf extracted with ethanol. Qualitative phytochemical analyses were accomplished by established methods. Antioxidant, antibacterial and cytotoxic effects were, respectively, measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method, disc diffusion method and brine shrimp lethality bioassay. The presence of alkaloids, glycosides, anthraquinone glycosides and flavonoids were detected in the leaf extract. The extract showed significant ($p < 0.01$) radical scavenging effect (IC_{50} , 200.96 ± 0.85 $\mu\text{g/ml}$) in comparison to ascorbic acid (IC_{50} , 1.24 ± 0.08 $\mu\text{g/ml}$). The extract also showed significant ($p < 0.05$) zone of inhibitions against Gram-positive *Bacillus cereus* (13 ± 1.05 mm) and *Bacillus megaterium* (9 ± 0.85 mm), and Gram-negative *Vibrio cholera* (20 ± 0.68 mm), *Escherichia coli* (8 ± 0.92 mm), *Pseudomonas aeruginosa* (15 ± 1.03 mm), *Salmonella paratyphi* (9 ± 0.79 mm) and *Shigella dysenteriae* (8 ± 0.88 mm). In cytotoxic assay, the extract showed the LC_{50} value 315.5 $\mu\text{g/ml}$ which was statistically significant ($p < 0.01$) compared to reference cytotoxic agent vincristine sulfate (LC_{50} , 38.99 $\mu\text{g/ml}$).

Keywords: *Cassia hirsuta*; DPPH radical scavenging; Antibacterial; Cytotoxic; LC_{50} Value; IC_{50} Value.

Introduction

The use of traditional medicines and plant drugs against various diseases receive considerable attention since last couple of decades (Kala, 2006) because they are believed to free from side effects (Parvath and Brindha, 2003), convenient in using, economic in costing and versatile in disease relief. A lot of indigenous medicines have therefore been using even since the ancient time (Abu-Rabia, 2005) with successful remedial outcome.

Cassia hirsuta is an evergreen shrubby herb (0.2-2.4 m high), pubescent or sometimes pilose with hairy leaves, that is rarely found in Chittagong especially in the hilly area, although it is widely distributed in Bolivia, Caribbean Jamaica, Cuba, Colombia, Ecuador, El Salvador, Peru and Surinam, Nigeria, east and central Africa. It is also found in some region of India and Myanmar. It has been used by the tribes of Chittagong hill tracts for the treatment of various diseases. No local 'Bengali' name of the plant is known, however, in Myanmar it is commonly known as 'Kanduak' (Mazumder *et. al.*, 2008) and in Nagaland as 'Khuku'.

Different parts of this plant have been used for stomach troubles, dysentery, abscesses, rheumatism, hematuria, fever and

other diseases. The root is pasted with cumin and taken internally to treat stomach burning after a meal. The leaves are successfully and commonly used to fix bone fractures and sprains in cattle and in humans. Oral administration of powdered dried seeds works against impotency and weakness (Jamir *et. al.*, 2010). Powdered seeds are also used in various dental complications (Revathi and Parimelazhagan, 2010; ; Singh and Singh, 2007). In India, 19 species of the genus *Cassia*, which are useful in various ways to mankind, are well documented and their distribution, identification keys and ethnomedical properties are provided. However, there is a lack of knowledge regarding the biological activities of *C. hirsuta*. This study explored the free radical scavenging effect, antibacterial and cytotoxic effect of *C. hirsuta* leaf ethanolic extract.

Materials and methods

Plant material

Plant materials were collected from Chittagong University Campus. The plant was taxonomically identified. A voucher specimen is preserved in Bangladesh National Herbarium with the accession No. BCUA- 03/12.

*Corresponding author. e-mail: atiarh@yahoo.com

Chemicals and reagents

Absolute alcohol and 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, Munich, Germany. Tetracycline disc (30 µg/disc, Oxoid, England) and ascorbic acid (BDH, England) were used as reference standards for antibacterial screening and free radical scavenging assay, respectively. Vincristine sulfate (Merck, Germany) was used as reference cytotoxic agent in brine shrimp lethality test.

Preparation of leaf extract

The fresh leaves of *C. hirsuta* were washed with deionized water immediately after collection. Air dried leaves were ground into powder (600 g) to be macerated in absolute ethanol for 7 days at room temperature (23 ± 5) °C. Extract was filtered through Whatman No. 1 filter paper (32cm) and concentrated under reduced pressure at the temperature below 50°C through rotatory vacuum evaporator (RE200, Sterling, UK). The crude extract (72 g blackish-green colored, 12% w/w yield) was stored at 4°C for further use.

Phytochemical screening

The plant extract was phytochemically screened for the qualitative detection of alkaloids, glycosides, cardiac glycosides, anthraquinone glycosides, terpenoids and flavonoids using standard techniques as described below:

Tests for alkaloids

The alkaloids test has been done according to the method described by El-olemy *et. al.* (1994). To conduct this test, a 0.5 g of extract was stirred with 5 ml 1% HCl on a steam bath and then the solution was cooled and filtered. 1ml of the filtrate was treated separately with drops of Mayer's, Hager's and Wagner's reagents and formation of dirty/dark brown, yellow-brown or reddish brown precipitate, respectively, indicated the presence of alkaloids.

Test for Glycoside

The presence of glycosides in the plant extract was confirmed by general tests as described by Sofowora (1984).

General test: A 500 mg of an ethanolic extract was dissolved in one ml of water. A few drops of aqueous NaOH solution were added to the solution. Development of yellow color indicated the presence of glycoside.

Test for cardiac glycosides

The presence of cardiac glycosides and O-glycosides in the plant extract was confirmed by Legal's test and Baljet tests

as described by Sofowora (1984).

- I Legal's test: A 100 mg of an ethanolic extract of the plant material was dissolved in two ml of pyridine and two ml of sodium nitroprusside solution (0.5%). The mixture was alkalined with NaOH (0.2 N) solution. Development of pink to red color development indicated the presence of cardiac glycoside due to lactone ring.
- II Baljet test: A drop of Baljet's reagent (95 ml 1% picric acid + 5 ml 10% NaOH) was added to a two ml of an ethanolic extract of plant material. Development of yellow orange color development indicates the presence of five membered lactone rings at C-17 of the aglycone in cardiac glycoside.

Test for anthraquinone glycosides

The presence of anthraquinone glycosides in the plant extract was confirmed by the following tests as described by Sofowora (1984):

- I O-glycoside test: Two ml of the extract was boiled with diluted HCl/concentrated H₂SO₄ (2-3 min) and filtered. Subsequently, after cooling and shaking with organic solvent (chloroform) organic layer was separated. Further shaking with NH₄OH (10% ammonia solution) the solution was allowed to keep standing for 30 min. The rose pink or cherry red or violate aqueous layer indicated the presence of anthraquinones.
- II C-glycosides test: The plant material (dry powder) was boiled with dilute HCl / FeCl₃ (1.0 g powder + 5 ml dilute HCl + 5 ml 5% FeCl₃, boiled for 5 min) and was allowed to cool. The mixture was then shaken with organic solvent (ether or benzene/chloroform) and then with NH₄OH or KOH. Positive result was indicated by rose red color in the aqueous alkaline layer.
- III Aglycone test: A 500 mg of leaf extract was shaken with 5 ml chloroform for at least 5 minutes and was filtered. Further shaking was done with NH₄OH (10% ammonia solution). A bright pink, red or violet color in the upper layer indicated the presence of anthraquinone.

Test for terpenoids

The presence of terpenoids in the plant extract was confirmed by the methods as described by Trease and Evans (1989). To perform this test, 0.5 g of extract and two mL of chloroform was mixed in a test tube and then three mL of

concentrated H₂SO₄ was added carefully to form a layer. A reddish brown color in the interface confirmed the presence of terpenoids in the extract.

Test for flavonoids

The presence of flavonoids in the whole plant extract was confirmed by NaOH and lead acetate tests as described by Harborne (1973).

- I General test: Small amount of an ethanolic extract of the plant material was added with a few drops of concentrated HCl. Immediate development of a red color indicated the presence of flavonoids.
- II Specific test: A 0.5 mL of an ethanolic extract was placed in a test tube and was added a small piece of magnesium or zinc ribbon. Then 5-10 drops of concentrated HCl was added and boiled for few minutes. Development of orange to red color indicated the presence of flavones. Development of red to crimson color indicated the presence of flavonols. Development of crimson to magenta color indicates the presence of flavanones. And occasionally a green or blue color indicates the presence of flavonoids.

Preparation of media

Müller-Hinton agar media [(Hi media, India), final pH 7.3±0.2 (at 25°C)], was used for antibacterial screening. Artificial seawater (3.8 % NaCl solution) has been used for brine shrimp lethality assay.

Assay for radical scavenging effect

The free radical scavenging effect of *C. hirsuta* leaf extract and ascorbic acid was assessed with the stable scavenger 1,1-diphenyl-2-picrylhydrazyl (DPPH) with slight modifications of the method described by Brand-William *et al.* (1995). Different concentrations (20, 50, 100, 200, 400, 600 and 800 µg/ml) of *C. hirsuta* leaf extract were prepared in ethanol. Ascorbic acid solution was made with the concentration between 1-100 µg/ml. DPPH solution (0.004 %) was prepared in ethanol and five ml of this solution was mixed with the same volume of extract and standard solution separately. These solutions were kept in dark for 30 min. The degree of DPPH-purple decolorization to DPPH-yellow indicated the scavenging efficiency of the extract. The absorbance of the mixture was taken at 517 nm using UV-Visible spectrophotometer (UV-VIS 1200, Shimadzu

Corporation, Japan). Ascorbic acid was served as a positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The scavenging activity against DPPH was calculated using the following equation: Scavenging activity (%) = [(A-B)/A] × 100, where *A* was the absorbance of control (DPPH solution without the sample), *B* was the absorbance of DPPH solution in the presence of the sample (extract/ ascorbic acid). Percent (%) scavenging effect was plotted against log concentration to obtain the inhibition concentration (IC₅₀) value using linear regression analysis.

Assay for antibacterial effect

In-vitro antibacterial potential was screened out by disc diffusion method (Bauer *et al.*, 1966), a qualitative to semi-qualitative test. The bacterial suspension turbidity adjusted to McFarland standard number 0.5, in Müller-Hinton Broth. With a sterile cotton swab bacterial culture was streaked on previously prepared Müller-Hinton agar plate. Dried and sterilized paper discs were treated separately with desired concentration of previously prepared ethanolic solution of leaf extract using a micropipette dried in air under aseptic condition and placed at equidistance in a circle on the seeded plate. The concentrations of the extract used were 2 mg/disc. These plates were kept for 4-6 hours at low temperature and the test materials diffuse from disc to the surrounding medium by this time. The plates were then incubated at 37°C for 18 hours. The diameter of zone of inhibition produced by the extract was then compared with standard antibiotic tetracycline disc. Each sample was used in triplicate for the determination of antibacterial activity. Blank disc impregnated with ethanol followed by drying off was used as negative control.

Assay for cytotoxicity effect

Cytotoxic effect of the extract was determined by Brine shrimp lethality assay as described by Meyer *et al.* (1982). Simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimps were collected from the Institute of Marine Science and Fisheries, University of Chittagong, Bangladesh and hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called nauplii. *C. hirsuta* leaf extract (30 mg) was dissolved in 3 mL of DMSO to prepare the solution of 20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000 µg/mL by serial dilution. Each concentration was tested in triplicate. A control containing five ml of DMSO solvent was

used for each solvent. The final volume of the solution in each test-tube was made up to five mL with seawater immediately after adding shrimp larvae. The test tubes were maintained under illumination. After 24 hours have elapsed, survivors were counted with the aid of a 3x magnifying glass. The LC₅₀ values were calculated from Probit Chart using "Microsoft Excel 2010".

Statistical analysis

All data are presented as mean \pm standard deviation (SD) and were analyzed by one-way Analysis of Variance (ANOVA) (SPSS for windows, version 18.0, IBM Corporation, NY, USA). The values were considered significantly different at $p < 0.05$.

Results and discussion

Results of phytochemical screening showed the presence of Alkaloids, glycosides, anthraquinone glycosides, flavonoids and terpenoids in the *C. hirsuta* ethanolic extract (Table I). These secondary metabolites are known to show medicinal and physiological activity while their occurrence and activity varies from plant to plant (Sofowora, 1984). The antioxidant activity (DPPH free radical scavenging activity) of *C. hirsuta* leaf extract in comparison to ascorbic acid has been shown in Fig. 1. Among seven different concentrations used in the study (20 to 800 $\mu\text{g/mL}$) (Fig. 1) the highest scavenging activity 78.70% was shown at concentration 800 $\mu\text{g/mL}$ and at the same concentration ascorbic acid showed 98.34%

scavenging activity. Percentage scavenging activity was plotted against log concentration. From the graph IC₅₀ (Concentration for 50% inhibition) values calculated by linear regression analysis for *C. hirsuta* extract and vitamin C were found to be 200.96 ± 0.85 and $1.24 \pm 0.08 \mu\text{g/mL}$, respectively.

Searching for natural anti-oxidant molecule is becoming very important in medical science as increasing amount of evidence suggests that oxidative stress is linked to pathophysiological mechanisms concerning multiple acute and chronic human diseases (Dalle-Donne *et al.*, 2006), obesity (Vincent *et al.*, 2007). But exposure to ROS is unavoidable because of the continuous endogenous generation of these agents by physiological processes, particularly mitochondrial respiration (Circu & Aw, 2010) and low levels of ROS are also indispensable in many biochemical processes, (intracellular messaging, cell differentiation, cell progression, arrest of growth, apoptosis (Ghosh *et al.*, 1998), immunity (Yin *et al.*, 1995), and defense against micro-organisms (Bae *et al.*, 1997, Lee *et al.*, 1998). Reactive oxygen and nitrogen species, ROS/RNS are essential to energy supply, detoxification, chemical signaling and immune function. Hence, the prevention of excess free radical formation is a vital step for cell survival (Valko *et al.*, 2006). Naturally there are several lines of antioxidant defense mechanisms. The first defense line is to inhibit the formation of active oxygen species and free radicals by sequestering metal ions, reducing hydroperoxides and hydrogen peroxide and to quench superoxide and

Table I. Observation and result of phytochemical screening

Phytochemicals	Name of the test	Observation	Result
Alkaloids	Mayer's test	Creamy white precipitate	+
	Hager's test	Yellow crystalline precipitate	+
	Wagner's test	Brown precipitate	+
Glycosides	General test	Yellow color	+
	Legal's test	No pink to red color	-
Cardiac glycosides	Baljet's test	No yellow orange color	-
	O-glycoside test	Rose pink in the aqueous layer	+
Anthraquinone glycoside	C-glycoside test	Rose red coloration in aqueous layer	+
	Aglycones test	Bright pink coloration	+
Terpenoids	Salkowsky test	No red color.	-
	General test	Rose pink in the aqueous layer	+
Flavonoids	Specific test	Orange to red color	+

The sign "+" indicates the presence, "-" indicates the absence.

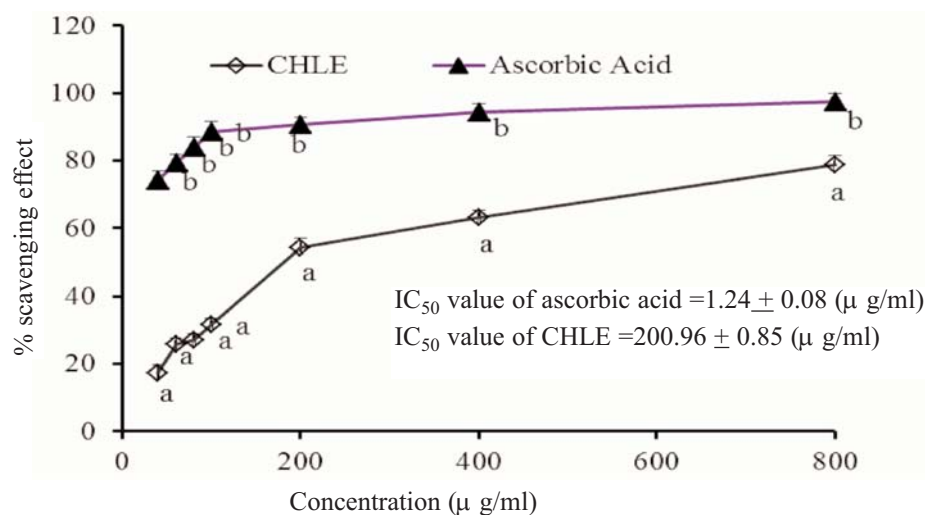


Fig. 1. DPPH free radical scavenging action of CHLE (*C. hirsuta* leaf extract) and ascorbic acid with the plotted concentration. Data are shown as mean \pm SD for triplicate of each concentration. Subscript letters, a & b, shown on the line graph indicate that the values are significantly different (Tukey multiple range *post hoc* test, $p < 0.05$) from each other.

singlet oxygen. The radical-scavenging antioxidants function as the second line defense. The third-line of defense is the repair, de novo and clearance of oxidative damaged lipids, proteins and DNA (Jebakumar *et al.*, 2012). There is another defense mechanism in which appropriate antioxidants are produced and transferred to the correct place at the correct time and in the correct amounts. In the present study the IC_{50} value of *C. hirsuta* ($200.96 \pm 0.85 \mu\text{g/ml}$) was found to be considerably higher as regards the IC_{50} value of Ascorbic Acid ($1.24 \pm 0.08 \mu\text{g/ml}$). It signposts the plants (*C. hirsuta*) second line antioxidant defense mechanisms, weakness as well as the possibility of being other antioxidant defense lines resiliency.

Antibacterial activity of *C. hirsuta* extract was evaluated on four Gram-positive and six Gram-negative bacteria by disc diffusion method using tetracycline as standard antibiotic disc. Plant extract showed zone of inhibition to almost all the strains (at dose 2 mg/disc) except *Bacillus subtilis*. Crude extract at the concentration of 2 mg/disc showed 13.0, 9.0 & 6.0 mm (Fig. 2) diameter zone of inhibition against Gram-positive *Bacillus cereus*, *Bacillus megaterium* & *Staphylococcus aureus*, respectively and 8.0, 6.0, 9.0, 15.0, 20.0, 8.0 mm (Fig. 2) diameter against Gram-negative *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Shigella dysenteriae*. On the other hand, standard antibiotic tetracycline showed significant antibacterial activity against all tested Gram-positive and Gram-negative bacteria. The activ-

ity of tested plant depends on their kind, mode of extraction and used species tested. The most common modes of action are interference with the cell membrane and cell wall, interference with nucleic acids, and enzyme interactions (Lambert and O'Grady, Neu, 1992). Results implicated that the Gram-negative bacteria were more sensitive to the extract than the Gram-positive bacteria (Fig. 2). Although the outer membrane (OM) of Gram-negative bacteria protects cells from many external agents, it is possible to weaken it specifically by various agents, collectively called permeabilizers, which disintegrates the LPS layer and increase the permeability of the OM to hydrophobic agents. Permeabilizers themselves may not be bactericidal, but they may potentiate the activity of other compounds, thus acting synergistically. So the observed anti-microbial effect could be combined for the action of permeabilizers and antimicrobial component of plant extract.

Bacillus cereus, *Pseudomonas aeruginosa* and *Vibrio cholerae* were the most susceptible bacteria, the observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by active principles of the extract of *C. hirsuta*. In contrast, *Staphylococcus aureus* and *Salmonella typhi* showed the least susceptibility to the extract whereas *Bacillus subtilis* showed no sensitivity to the extract. This may be due to the fact that these organisms have intrinsic resistance from a restrictive outer membrane barrier and transenvelope

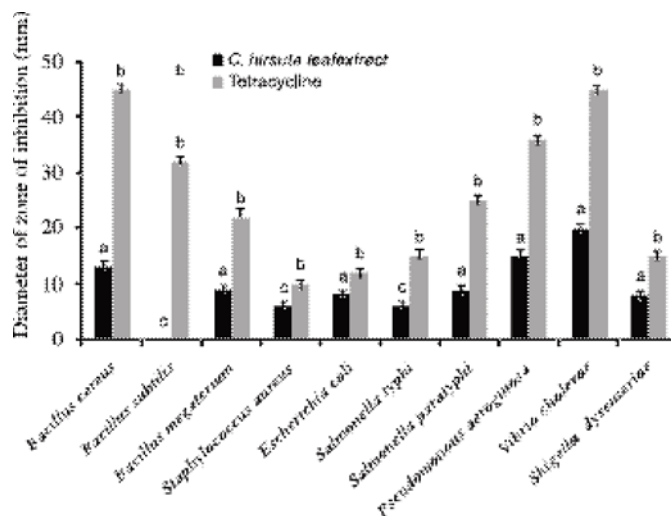


Fig. 2. Comparative antibacterial effect of *C. hirsuta* leaf extract and tetracycline expressed as zone of inhibition (diameter in mm). Data are shown as mean \pm SD for triplicate of concentration. ^{a,b&c}Superscript letters shown in the bar line indicate that the values are significantly different (Tukey multiple range, *post hoc test*, $p < 0.05$) from each other (** $p < 0.01$, SPSS for windows, version 18.0)

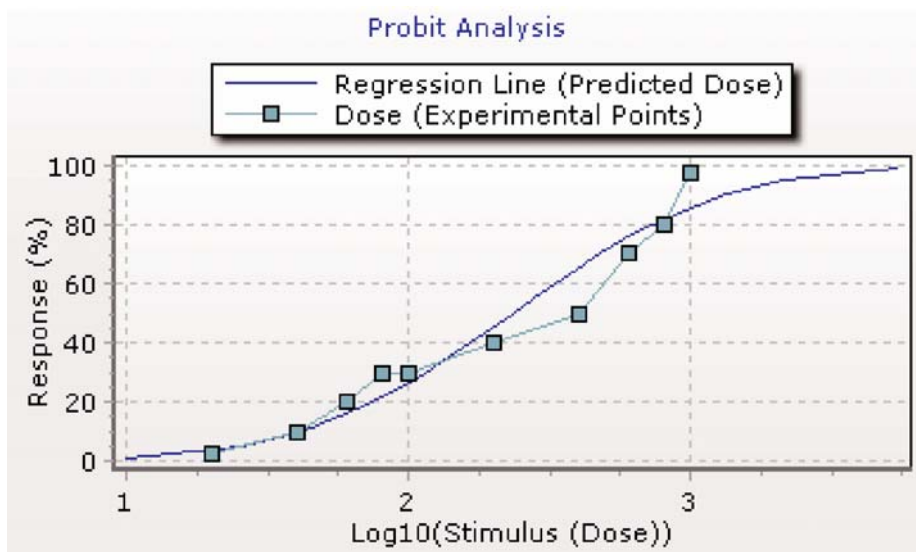


Fig. 3. Probit analysis at 95% confidence limit ($\mu\text{g/ml}$) with regression value for brine shrimp treated with *C. hirsuta* (Extract) and vincristine sulfate (VS)

multidrug resistance pumps (Girish and Satish, 2008). The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Mohanasundari *et al.*, 2007; Britto, 2001).

Therefore, the present study justifies the claimed uses of *C. hirsuta* in the traditional system of medicine to treat various infectious diseases caused by the microbes.

Brine shrimp lethality is a general bioassay which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions (McLaughlin *et al.* 1991). In toxicity assessment of plant extracts by brine

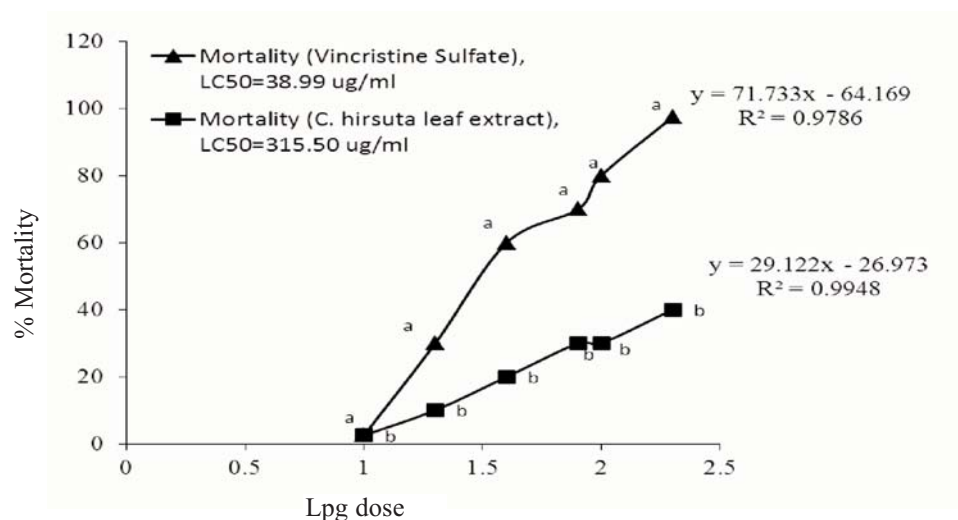


Fig. 4. Lethality of *C. hirsuta* leaf extract against 24-hour-age brine shrimp (*A. salina*) in comparison to standard vincristine sulfate with the plotted concentration. Data are shown as mean \pm SD of twenty shrimps for each concentration. Subscript letters, a & b, shown in the plot indicate that the values are significantly different (Tukey multiple range *post hoc test*, $p < 0.05$) from each other (** $p < 0.01$, SPSS for windows, version 18.0)

shrimp bioassay, an LC_{50} value lower than 1000 $\mu\text{g}/\text{mL}$ is considered bioactive (Meyer *et al.*, 1982). In this bioassay, a dose dependent lethality was observed for different concentrations of extract used to determine the cytotoxicity by brine shrimp. However, probits were plotted against corresponding log concentration of extract and from the plot LC_{50} (Lethal Concentration 50) value was calculated by regression analysis (Fig. 3). In the present investigation the LC_{50} value of the extract and standard compound (vincristine sulfate) was found to be 315.5 & 38.99 $\mu\text{g}/\text{mL}$ (Fig. 4) respectively. The plant extracts in this investigation possess different types of secondary metabolites alkaloids, flavonoids, glycosides or saponins etc. (Table I) which are well known for possessing toxicological and pharmacological properties. The cytotoxicity of plant material is considered to be due to the presence of antitumor compounds (Kawsar *et al.*, 2008). The correlation between the brine shrimp lethality assay and *in vitro* growth inhibition of human solid tumor cell lines shows the value of this bioassay as a pre-screening tool for anti-tumor drug research (Anderson *et al.*, 1991). In our test the crude extract of *C. hirsuta* was found to be bioactive.

Conclusion

This study demonstrates the potentials of the *C. hirsuta* extract in free-radical scavenging, bacterial inhibition and cytotoxicity. Antibacterial and cytotoxic effect of the extract lead to the possession of different active phytochemicals in this extract and their individual or synergistic function could also be delineated from this study. Future study is required to

isolate and characterize the individual bioactive compounds as well as to elucidate the possible mechanisms lying with these effects.

References

- Abu-Rabia A (2005), Urinary diseases and ethnobotany among pastoral nomads in the Middle East. *J. of Ethnobiol. and Ethnomedi.* **1**: 4.
- Anderson JE, Wenwen MA, Smith, LD, Chang CJ and McLaughlin JL (1992), Biologically active β -lactones methylketoalkenes from *Lznderabenzozn*. *J. Nat. Prod.* **55**: 71-83.
- Bae YS, Kang SW and Seo MS (1997), Epidermal growth-factor (EGF)-induced generation of hydrogen peroxide. *J. Biol. Chem.* **272**: 217-221.
- Bauer AW, Kirby WMM, Sherris JC and Turck M (1966), Antibiotic susceptibility testing by a standardized single disc method. *Ame. J. of Clini. Pathol.* **45**(4): 493-96.
- Brand-Williams W, Cuvelier ME and Berset C (1995), Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology.* **28**: 25-30.
- Britto JS (2001), Comparative antibacterial activity study of *Solanum incanum* (L.). *The Journal Swamy Botanical Club.* **18**: 81-82.

- Dalle-Donne I, Rossi R, Colombo R, Giustarini D and Milzani A (2006), Biomarkers of oxidative damage in human disease. *Clin. Chem.* **52**: 601-623.
- Circu ML and Aw TY (2010), Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radical Biology & Medicine.* **48**: 749-762.
- El-Olemy MM, Al-Muhtadi FJ and Afifi AA (1994), Experimental Phytochemistry. A Laboratory Manual. (King Saud University Press, KSA) 1994, pp 8-9.
- Girish HV and Satish S (2008), Antibacterial activity of important medicinal plants on human pathogenic bacteria -a comparative analysis. *World Applied Sciences Journal.* **5**(3): 267-271.
- Ghosh J. and Myers CE (1998), Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. *Proc. Natl. Acad. Sci.* **95**: 13182-13187.
- Harborne JB (1973), Phytochemical Methods: A guide to Modern Techniques of Plant Analysis. 1st Edn. (Chapman and Hall Ltd. London) 1973, pp 60.
- Jamir NS and Limasemba T (2010), Traditional Knowledge of Lotha-Naga tribes in Wokha District, Nagaland. *Indian Journal of Traditional Knowledge.* **9**(1): 45-48.
- Jebakumar AZ, Nondo HS, George SK and Manoj G (2012). Natural anti-oxidant and in-vitro methods for anti-oxidant activity. *International Journal of Pharmacology Research.* **2**(1): 46-55.
- Kala CP (2006), Ethnobotany and ethnoconservation of *Aeglemarmelos* (L.). *Indian Journal of Traditional Knowledge.* **5**(4): 537-540.
- Kawsar SMA., Huq E and Nahar N (2008), Cytotoxicity Assessment of the Aerial Parts of *Macrotylom auniflorum* (L.). *International Journal of Pharmacology.* **4**(4): 297-300.
- Lambert HP and O'Grady, FW (1992), Antibiotic and Chemotherapy, 6th ed. Churchill, Livingstone, Medical Division of Longman group U.K. Ltd., pp.72-135, 191-230.
- Lee YJ, Galoforo SS, Berns CM, et al. (1998), Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J. Biol. Chem.* **273**: 5294-5299.
- Mazumder PM, Percha V, Farswan M and Upaganlawar A (2008), Cassia: A Wonder Gift to Medical Sciences. *International Journal of Community Pharmacy.* **1**(2): 16-38.
- McLaughlin JL, Chang CJ and Smith DL (1991), Bench-Top Bioassays for the Discovery of Bioactive Natural Products: An Update. In: Studies in Natural Products Chemistry, Rhaman, AU (Ed.) Oxford, Elsevier. pp. 383-409.
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL (1982), Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Medica.* **45**: 31-34.
- Mohanasundari C, Natarajan D, Srinivasan K Umamaheswari S and Ramachandran A (2007), Antibacterial properties of *Passiflora foetida* (L.) a common exotic medicinal plant. *Afr. J. Biotechnol.* **6**(23): 2650-2653.
- Parvath S and Brindha R (2003), Ethnobotanical Medicines of Animalai union. *Ancient Sci. Life.* **22**: 14.
- Revathi P and Parimelazhagan T (2010), Traditional Knowledge on Medicinal Plants Used by the Irula Tribe of Hasanur Hills, Erode District, Tamil Nadu, India. *Ethnobotanical Leaflets.* **14**: 136-60.
- Singh I and Singh VP (2007), Antifungal properties of aqueous and organic solution extracts of seed plants against *Aspergillus flavus* and *Aspergillus niger*. *Phytomorphology.* **50**: 151-157.
- Sofowora A (1984), Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Nigeria. 1st Edn. pp150-153 and 162-172.
- Trease GE and Evans WC (1989), Pharmacognosy. 11th Edn. Brailliar Tiridel and Macmillian publishers, London.
- Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M (2006), Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* **160**: 1-40.
- Vincent HK, Innes KE and Vincent KR (2007), Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes. Metab.* **9**(6): 813-839.
- Yin GY, Yin YF and He XF (1995), Effect of zhuchun pill on immunity and endocrine function of elderly with kidney-yang deficiency. *Chung Kuo Chung Hsi I Chieh Ho TsaChih.* **15**: 601-603.

Received: 05 April 2012; Revised: 21 October 2012;
Accepted: 22 October 2012 .