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Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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## Full Length Research Paper

# Antibacterial and antifungal screening of extracts from six medicinal plants collected in Kinshasa-Democratic Republic of Congo against clinical isolate pathogens

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Results from the *in vitro* evaluation of the antibacterial and antifungal activities of six plant extracts indicated that the aqueous and the methanol extracts of *Acalypha wilkesiana* leaves and *Ageratum conyzoides* leaves exhibited good and efficient antifungal activity against *Candida albicans* with minimum inhibitory concentration (MIC) of 31.25 µg/ml and minimum fungicidal concentration (MFC) of 62.5 µg/ml while the methanol extract from *Pentaclethra macrophylla* inhibited the yeast growth with minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of 62.5 and 125 µg/ml respectively. The aqueous extracts from *Acalypha wilkesiana*, *Ageratum conyzoides*, *Bucchozia tholoniana* seeds, *Gulboutia demeusei* root and the aqueous and methanol extracts from *Pentaclethra macrophylla* stem bark showed good antibacterial activity against *Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Salmonella thyphimurium*, *Stahylococcus aureus* and *Shigella flexneri* with minimum inhibitory concentration (MIC) of 62.5 µg/ml and minimum bactericidal concentration (MBC) of 125 µg/ml according to the case. These results partly support and justify the traditional use of these plant extracts for treating infections in traditional medicine.

**Key words:** Plant extracts, antibacterial, antifungal, infections.

## INTRODUCTION

Bacteria's are responsible for serious and various human and animal infections. For example, *Pseudomonas*

*aeruginosa* is the most common bacteria in clinics with significant percentage of acquired infections (Abu-

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Shanab et al., 2004). *Sataphylococcus aureus* causes skin lesions which are often superficial, localized abscesses and food contamination or poisoning (Lotifpour et al., 2008). Intestinal disorders are caused by bacteria such as *Vibrio cholerae*, *Escherichia dysenteriae*, *Escherichia coli* and *Salmonella* species especially diarrhoea as a major cause of mortality and morbidity in developing countries (Chitemerere and Makangangama, 2011). Long before mankind the existence of microbes was discovered, the idea that some medicinal plants had antibacterial and antifungal potential since they contain active constituents belonging to different phytochemical groups, was well accepted (Rios; Recio, 2005). Thus, the therapeutic properties of various medicinal plants have been known to treat particularly human and animal infectious diseases as it is estimated between 60 to 90% of the population in developing countries turn to traditional medicine and consider it to be a normal part of its primary healthcare (WHO, 2002).

The rapid spread of bacteria and fungi expressing multidrug resistance (MDR) has encouraged the discovery of new antibacterial and antifungal agents. The trend use in alternative and complementary healthcare has prompted scientists to investigate various biological activities of medicinal plants mainly according to their uses in traditional medicine to prove their effectiveness and safety to support and justify their traditional uses. Crude extracts prepared from medicinal plants as infusion, decoction, macerate, tincture or powder are traditionally used by the population to treat various diseases particularly infections from different origins. Although their efficacy and mechanism of action have not been scientifically elucidated in most cases, these different medicinal preparations often give beneficial response to human and animal due to the presence of active compounds (Barnes et al., 2007).

In this present study, six medicinal plants belonging to different botanical families were selected and evaluated *in vitro* for their potential antibacterial and antifungal properties. All selected plant parts are used in traditional medicine in Kinshasa-Democratic Republic of Congo (DR Congo) to treat various ailments such as diarrhoea, wounds, burns and skin infections (Kerharo and Adam, 1974), (Oliver Bever, 1986), (Kambu, 1990), (Neuwinger, 2000). To treat wounds and burns, powder or decoction from the plant part is applied and this process gives good results as indicated by the acceleration of the rapid healing of affected part of the body and the decoction is employed to treat bacterial diarrhoea. For this study, the aqueous and methanol extracts from the selected plant parts were tested against 9 clinical isolate pathogens and 1 yeast *in vitro*. Their minimum inhibitory concentrations were determined by dilution method when compared to the reference antibacterial and antifungal products.

## MATERIALS AND METHODS

### Selected Plants

They include leaves of *Acalypha wilkensis* Mull. Arg. (Euphorbiaceae), leaves of *Ageratum conyzoides* L. (Asteraceae), seeds of *Buchholzia tholoniana* Hua (Capparidaceae), root of *Gulbourtia demeusei* (Harms) J. Leonard (Fabaceae), stem bark of *Pentaclethra macrophylla* Benth (Mimosaceae) and leaves of *Syzygium guineense* (Wild.) DC. var. *guineense* (Myrtaceae) collected in Kinshasa-Democratic Republic of Congo (DR Congo) in April 2011. All plants were identified by Mr Nlandu Lukebiako, B. of the Institute National d'Etudes et de Recherches en Agronomie, Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of each plant NL01042011AW, NL02042011AC, NL03042011BT, NL04042011GD, NL05042011PM and NL06042011SG for *A. wilkensis* leaves, *A. conyzoides* leaves, *B. tholoniana* seeds, *G. demeusei* root, *P. macrophylla* stem bark and *Syzygium guineense* leaves respectively has been deposited in the herbarium of this institute. All plant parts were dried at room temperature and reduced to powder.

### Preparation of extracts

45 g of each dried plant material were macerated with distilled water or methanol (200 ml each) during 24 h. After, each mixture was filtered and each filtrate evaporated in vacuum yielding corresponding dried extracts (Table 1).

### Phytochemical screening

This study was performed by thin-layer chromatography (TLC) on precoated silica gel plates F<sub>254</sub> (thickness later 0.25, mm, Merck, Germany) using different reagents and mobile phases described in the literature for the identification of major chemical groups such as alkaloids, anthraquinones, coumarins, flavonoids, terpenes and steroids. Hydrochloride acid 2M/*n*-butanol (heating with HCl 2 M for 40 min at 100°C and extracting the red color with iso-amylic alcohol, froth test and Stiasny's reagent (formol + HCl conc.) were used to detect anthocyanins, saponins and tannins respectively (Harborne, 1998).

### Antibacterial and antifungal testing

#### Selected test microorganisms

The selected microorganisms included the yeast *C. albicans*, and the bacteria *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Shigella flexneri*, *S. typhimurium* and *S. aureus*. The yeast and all bacteria were clinic isolated from patients diagnosed with infections in the Cliniques Universitaires du Mont-Amba/Kinshasa-DR Congo.

#### Culture medium

Bacteria and fungi were maintained on trypticase soy broth (TSB) plates and Sabouraud dextrose agar plates at 4°C respectively. Yeast were cultured in liquid Sabouraud dextrose medium for 48 h at 24 °C. Test inocula of fungi were prepared by harvesting matured sporulating cultures in Sabouraud agar broth. For inoculation with the yeast, homogenized mycelia cultures of two weeks old were

**Table 1.** Uses in traditional medicine of ethnopharmacologically selected medicinal plants

Plant	Uses
<i>Acalypha wilkesiana</i> Mull. Arg. (Euphorbiaceae)	The leaves are used to treat fungal skin diseases and function infections, pityriasis vesicolor, superficial mycoses. Associated to <i>Ocimum gratissimum</i> against methicillin resistant bacteria, to combat constipation, naso-pharyngeal infections, pain-killers, applied externally as an antiseptic to ulcers and wounds, anti-diarrhoeal agent, used as antibiotic, bacteriostatic and fungistatic. The plant possess anticancer, antibacterial, antifungal, antidiabetic, analgesic, antiparasitic and antihypertensive properties and so on (Neuwinger, 2000; Lim et al., 2013; Royal Botanic Gardens Kew, 2014).
<i>Ageratum conyzoides</i> L. (Asteraceae)	The grain cannot be used if it is contaminated due to its toxicity. Leaves and entire plant are used to treat colic, colds and fever, diarrhea, rheumatism, spasms, headache, pneumonia, wounds, burns, and as a tonic. They are also recommended to treat burns, wounds infectious conditions and bacterial infections. The leaf sap is used to treat conjunctivitis, as galactagogue, liver and stomach problems, otitis, and so on (Anonymous, 2014).
<i>Bucchozia tholoniiana</i> Hua (Capparaceae)	The seed is used to treat inflammation, gynecological infections, migraine, cough and stomach pains (Oliver Bever, 1986).
<i>Gulbourtia demeusei</i> (Harms) J. Léonard (Fabaceae)	The stem bark is employed to treat otitis, wound, skin infections and diarrhea (Oliver Bever, 1986)
<i>Pentacletra macrophylla</i> Benth. (Euphorbiaceae)	The bark is employed as a decoction to treat abdominal pains, dysmenorrhea, urogenital infections, gonorrhoea, stomach pains, dysentery, hernia, as anthelmintic, The ripe seeds and stem bark powder are applied externally to treat wounds, and burns, Extracts of the leaf, stem bark, seeds and seed pulp have anti-inflammatory and antihelmintic properties. They are used to treat gonorrhoea and convulsions, and as analgesic. The root and stem barks are used as a laxative, enema against dysentery and liniment against itch. It is also used to treat stomach pains, wounds, burns asthma, abscess, heart pains, diarrhoea and ulcerations, and so on (Kerharo and Adam, 1974; Kambu, 1990; Neuwinger, 2000).
<i>Syzygium guineense</i> (Wild.) DC. (Myrtaceae)	The stem bark or leaf have febrifuge, anthelmintic and purgative properties They also are used to treat menstrual cycle, stomach pains, colics, dysentery, rheumatism pains, malnutrition, delirium, naso-pharyngeal affections, pain killers, pulmonary troubles and diarrhoea. The leaf powder is a healing agent. An infusion of bark and roots soaked in hot water is used as a purgative, the bark can be toxic and deaths from its use have been recorded. The root is soaked in water for drinking and bathing to treat epilepsy, Root extracts are used as taeniocides. Bark decoction is employed against stomach-ache, diarrhoea and malaria, as a mildly laxative and applied in draught or in baths as a tonic. Its infusion is taken against coughs, asthma, throat problems and intercostal pains. The bark is used for the treatment of snakebites and so on (Kerharo and Adam, 1974; Maroyi, 2008; Kupumba, 2014).

Kerharo and Adam, 1974, Kambu, 1990, Neuwinger, 2000, Anonymous, 2014; Maroyi, 2008. Lim et al., 2013; Kupumba, 2014, Royal Botanic Gardens Kew, 2014.

used (Vanden Berge and Vlietinck, 1991; Cimanga et al., 1998).

#### Antibacterial and antifungal testing

The antimicrobial and antifungal tests extracts used against bacteria and yeast respectively were performed by the microtiter plate dilution method. For this technique, 2 mg of each extracts was dissolved in dimethylsulfoxide (DMSO), water and trypticase soy broth (TSB), final volume 2 ml (concentration of DMSO < 1%) to have a final concentration of 1 mg/ml. These stock solutions were repeatedly diluted in two fold with TSB or Sabouraud medium to obtain a series of test concentrations samples from 500 to 10 µg/ml.

An inoculum consisting of about 10<sup>5</sup> microorganisms/ml TSB or 10<sup>6</sup> yeast /ml Sabouraud medium was incubated overnight at 37°C for 24 h. A 1/1000 dilution of each suspension was prepared with the corresponding medium. Thereafter, 1 ml of the test sample was added. Each vertical column contained 100 µl TSB and bacteria or 100 µl Sabouraud medium and yeast without test sample was in control for the normal growth of bacteria or yeast. The plates were incubated at 37°C in humidified atmosphere for 24 h. The inhibition of bacterial and yeast growth was evaluated by comparing it with normal bacterial or yeast growth in control holes prepared without test samples. The MIC was determined as the lowest concentration of the sample that completely inhibited macroscopic growth of bacteria or yeast. To determine the minimum bactericidal or

**Table 2.** Amount plant extracts (g) from 45 g of plant material

Plant species	Part used	Aqueous extract (%)	Methanol extract (%)
<i>Acalypha wilkesiana</i> Mull. Arg	L	1.94 (4.31)	3.94 (8.76)
<i>Ageratum conyzoides</i> L.	L	4.5 (10)	4.4 (9.98)
<i>Buchholzia tholoniana</i> Hua	F	1.37 (3.04)	2.70 (6.00)
<i>Guilourtia demessii</i> (Hams) J. Leonard	R	3.07 (6.82)	7.27 (16.16)
<i>Pentaclethra macrophylla</i> Benth.	Sb	0.8 (1.78)	6.25 (13.89)
<i>Syzygium guineense</i> (Wild) D.C.	L	1.44 (3.20)	7.20 (16.61)

L: leaf, R: root, Sb: stem bark

**Table 3.** Phytochemical screening of the aqueous extracts of selected plant parts

Chemical groups/Plant extract	AC	AW	BT	GD	PM	SG
Alkaloids	++	++	-	++	++	-
Anthraquinones	-	-	+	-	+	-
Anthocyanins	-	-	-	-	-	-
Coumarins	++	-	+	-	-	-
Flavonoids	+	++	+	+	+	+
Tannins	+	+	-	-	++	++
Terpenes/steroids	++	++	++	++	++	++
Saponins	-	-	-	+	+	+
Sugars	-	-	+	+	+	+

These chemical groups were also detected in each corresponding methanol extract. AC: *Ageratum conyzoides*, AW: *Acalypha wilkesiana*, BT: *Buchholzia tholoniana*, GB: *Guilourtia demesei*, PM: *Pentaclethra macrophylla*, SG: *Syzygium guineense*.

fungicidal concentration (MBC or MFC), the two lowest concentrations which inhibited bacterial or yeast growth were plated out on a nutrient agar and incubated at 37°C for 24 h. Results were evaluated by comparing them with the control holes containing bacteria or yeast without test sample (Vanden Berghe and Vlietinck, 1991; Cimanga et al., 1998).

## RESULTS AND DISCUSSION

The type and level of different biological activities exhibited by any plant extract is dependent on many factors such as geographical area, time of collection, soil conditions, harvesting time, moisture content, drying method, storage conditions and post-harvest process (Wendakoon; Calderon, 2012). Also, the relatively high temperature and humidity can be generated during tissue grinding and can denature chemical constituents, the extractive solvent can also affect the level and composition of secondary active metabolites extracted. It is well known that the bacteriostatic effect of an antibiotic is sufficient to treat infections in correlation with the host's defense such as cellular and humoral systems while severe infections necessitate a rigorously bactericidal or

fungicidal agent administered together which may produce a synergistic effect (Hardman and Limbird, 2001; Wheels, 2008). In the present study, Table 1 reports some traditional uses of the six ethnopharmacologically selected medicinal plants while Table 2 shows the amounts of the aqueous and methanol extracts of each treated plant part. The six medicinal plants chosen for this study are commonly used for the treating diverse infections in traditional medicine in Kinshasa according to the daily practices of interviewed traditional healers and the literature data. Some of them are known to produce bioactive products (Djoukeng et al., 2005). Table 3 reports results from the phytochemical screening of the aqueous extract of all selected plant parts. The chemical composition of each methanol extract was similar to that of the corresponding aqueous extract. These results revealed the presence of alkaloids in *Guilourtia demesei*, *Pentaclethra macrophylla* and *Syzygium guineense* extracts, flavonoids, steroids and terpenes in all plant extracts, tannins in *Ageratum conyzoides*, *Acalypha wilkesiana*, *Pentaclethra macrophylla* and *Syzygium guineense*, saponins in *Guilourtia demesei*, *Pentaclethra macrophylla* and *Syzygium guineense*

extracts and sugars in *Buccholzia tholoniana*, *Guibourtia demeusei* and *Pentaclethra macrophylla*. Coumarins were only detected in *Buccholzia tholoniana* while anthocyanins were not found to be present in all extracts in our experimental conditions.

The dilution method used in this study has been currently employed in other previous investigations (Kambu et al., 1990; Vlietinck et al., 1995; Fabry et al., 1998; Cimnaga et al., 1998; David et al., 2009; Singla et al.; 2011, Djeussi et al., 2013) and is recommended as a good method for determining the relative potency of crude extracts and establishing their antibacterial and antifungal spectrum as it facilitates the use of different strains and to determine their MIC and MBC or MFC. It concerns polar, apolar and complex extracts in testing to establish their real potency by determining their MIC and MBC or MFC (Rios; Recio, 2005). Beside the dilution procedure, the disc diffusion method is also frequently use with high test concentrations from 1 to 100 mg to evaluate antibacterial and antifungal activity of plant extracts, it is only a qualitative technique since the MIC and MBC are not determined (Kelmanson et al., 2000; Samy and Ignacimuthu, 2000; Islam et al., 2008; David et al., 2009, Jamuna Bai et al., 2011; Chitemerere et al., 2011; Muhamed Muback et al., 2011; Neeraj and Madivi, 2011; Debajit et al., 2012; Israr et al., 2012; Sakunpak and Panichayupakaranant, 2012; Sharmeen et al., 2012; Tabasum et al., 2013; Gupta et al., 2013). To avoid this inconvenience, both techniques are used at the same time (Jagessar et al., 2008; Oskay et al., 2009; Chitemerere and Mukanganyama, 2011; Kondo et al., 2010; Shinkafi and Dauda, 2013; Raghavendra and Mahadevan, 2011; Dugler and Dugler, 2012; Sadeghi-Nejad and Azish, 2013) Antibacterial and antifungal activities of plant extracts or other compounds is largely influenced by the susceptibility testing method used (Vanden Berghe and Vlietinck, 1991).

For the present study, the following criteria were adopted to appreciate the level of evaluated activities: MIC, MBC or MFC  $\leq 10$   $\mu\text{g/ml}$ : pronounced activity,  $10 \leq$  MIC, MBC or MFC  $< 100$   $\mu\text{g/ml}$ : good activity,  $100 \leq$  MIC, MBC or MFC  $\leq 125$   $\mu\text{g/ml}$ : moderate activity,  $125 <$  MIC, MBC or MFC  $\leq 250$   $\mu\text{g/ml}$ : low activity,  $250 <$  MIC, MBC or MFC  $\leq 500$   $\mu\text{g/ml}$ : very low activity, MIC, MBC or MFC  $> 500$   $\mu\text{g/ml}$ : inactive. Results presented in Table 4 shows the MICs of tested plant extracts. They show that the aqueous and the methanol extracts from all selected medicinal plants exhibited antibacterial (bacteriostatic) and antifungal (fungistatic) activity at different extents.

When tested against the yeast *C. albicans*, the most active extracts with good activity were the aqueous extracts from *A. wilkesiana* leaves and *A. conyzoides* leaves inhibiting the yeast growth activity with MIC value of 31.25  $\mu\text{g/ml}$ . The methanol extracts from *A. wilkesiana*,

*A. conyzoides* and *Pentaclethra macrophylla* stem bark also presented good antifungal activity with MIC value of 62.5  $\mu\text{g/ml}$ . The aqueous extracts of *Buccholzia tholoniana* seeds and *P. macrophylla*, and the aqueous and methanol extract of *Syzygium guineense* leaves showed moderate activity with MIC value of 125  $\mu\text{g/ml}$ . The remaining samples displayed low, very low antifungal activity or were inactive against the selected yeast (Table 4). The observed effect of these plant extracts against *C. albicans* was fungistatic at different degrees. Results in Table 4 also show the effect of these extracts against 9 clinical isolate pathogens. They indicated that the aqueous from *A. conyzoides* exhibited good antibacterial activity (bacteriostatic effect) against *E. coli* with MIC value of 62.5  $\mu\text{g/ml}$ . The same minimum inhibitory concentration was also presented by the methanol extracts from *P. macrophylla* and *S. guineense* against *Enterobacter* species, the aqueous extracts from *A. wilkesiana* and *P. macrophylla*, and the methanol extract from *P. macrophylla* against *Klebsiella oxytoca*, the aqueous extracts from *A. wilkesiana*, *G. demeusei*, *P. macrophylla* and the methanol extract of this last plant against *Proteus mirabilis*, the aqueous extracts from *A. conyzoides* and *P. macrophylla* against *Salmonella thyphimurium*, the aqueous extract of *B. tholoniana* against *S. aureus*. The remaining samples were found to display moderate activity (MIC = 125  $\mu\text{g/ml}$ ), low ( $125 <$  MIC  $\leq 250$   $\mu\text{g/ml}$ ), very low ( $250 <$  MIC  $\leq 500$   $\mu\text{g/ml}$ ) or were inactive (MIC  $> 500$   $\mu\text{g/ml}$ ) against the selected clinical isolate bacteria (Table 4).

Table 5 shows the minimum bactericidal (MBCs) and fungicidal (MFCs) concentrations of the selected plants extracts. Results indicated that the aqueous extracts from *A. wilkesiana* and *A. conyzoides*, and the methanol extract from *P. macrophylla* presented good fungicidal activity with MBC value of 62.5  $\mu\text{g/ml}$  against *C. albicans*. The aqueous and methanol extract from *P. macrophylla* exhibited good activity against *K. oxytoca* (MBC = 62.5  $\mu\text{g/ml}$ ). The same MBC was also shown by the aqueous extracts of *A. wilkesiana*, *G. tholoniana*, the aqueous and methanol extract of *P. macrophylla* against *P. mirabilis*, the aqueous extract of *A. conyzoides* and *P. macrophylla* against *S. thyphimurium*. The remaining plant extracts showed moderate, low and very low bactericidal and fungicidal activities or were devoid with these effects according to the case (Table 5). The bacteriostatic and bactericidal effect of the aqueous extracts of *A. conyzoides*, *A. wilkesiana* and *G. demeusei*, and the methanol extract of *P. macrophylla* was higher compared to that of norfloxacin, used an antibiotic reference product against some bacteria such as *E. coli*, *E. cloacae*, *K. oxytoca*, *P. mirabilis*, *S. thyphimurium*, *S. aureus* and *S. flexneri* according to the tested bacteria. The activity of the remaining extracts was sometime similar or lower



**Table 4.** Antibacterial and antifungal activities of plant extracts (MIC, µg/ml)

Extract	Microorganism								
	C. a.	Es. c.	En. c.	K. p.	K. o	P. m.	S. f.	S. a.	S. t.
<b><i>A. wilkesiana</i></b>									
Aqueous	31.1	125	125	250	62.5	62.5	125	250	125
Methanol	62.5	250	250	250	125	125	500	125	500
<b><i>A. conyzoides</i></b>									
Aqueous	31.5	62.5	125	250	250	125	62.5	250	125
Methanol	62.5	125	250	125	250	250	125	250	250
<b><i>B. thioniana</i></b>									
Aqueous	125	1000	500	125	250	500	250	62.5	1000
Methanol	1000	1000	500	250	500	500	250	125	250
<b><i>G. demeussi</i></b>									
Aqueous	500	125	500	250	125	62.5	125	125	62.5
Methanol	500	250	250	250	250	125	250	250	125
<b><i>P. macrophylla</i></b>									
Aqueous	125	125	125	125	62.5	62.5	62.5	125	125
Methanol	62.5	125	62.5	250	62.5	62.5	125	125	250
<b><i>S. guineense</i></b>									
Aqueous	250	500	125	500	125	125	500	500	250
Methanol	125	500	62.5	500	250	250	500	500	250
Norfloxacin	-	125	62.5	250	500	125	250	125	125
Tetracycline HCl	-	7.81	7.81	3.90	3.90	1.95	0.97	0.98	0.97
Nystatin	0.25	-	-	-	-	-	-	-	-

Legend: C.a: *Candida albicans*, Es.c: *Escherichia colis*, En.c: *Enterobacter cloacae*, K.p: *Klebsiella pneumonia*, K.o: *Klebsiella oxytoca*, P.m: *Proteus mirabilis*, S.f: *Schigella flexneri*, S.a: *Staphylococcus aureus*, S.t: *Staphylococcus thymurium* -: no tested.

and 5). According to Bauernfeind and Petermüller (1983) and Boyd et al. (2009), MICs of norfloxacin for isolate pathogens from hospital ranged from 0.03 to 0.15 µg/ml. Results from our study showed that this antibiotic had high MICs against all clinical isolated bacteria tested in the present study. This finding suggested that these bacteria are resistant to norfloxacin. Tetracycline used also as an antibiotic reference product presented high antibacterial activity than norfloxacin and all tested extracts. However, the antifungal and fungicidal activity of all tested extracts was lower than that of nistatin used as an antifungal reference product (Tables 4 and 5).

Our results are only qualitatively in good agreement with those previously reported by Kambu et al. (1990) and Tona et al. (1999) concerning the antibacterial activity of *P. macrophylla* stem bark, the antibacterial and antifungal

activity of *A. conyzoides* leaves described by Ahmagboul et al.,(1985), Yamamoto et al., (1991), Gamesan and Krishnaraju (1995), Dayie et al., (2008) and Okwori et al. (2013) and the antibacterial and antifungal activity of *A. wilkesiana* leaves reported by Alade and Irobi (1993); Adesina et al., (2000); Gotep et al., (2010) and Haruna et al. (2013). Although these activities were carried out by the diffusion method. Gallic acid, corilagin and geraniin isolated from the leaves were reported to be responsible for the observed antibacterial activity (Adesina et al., 2000). In clinical trial on dermatitis, the water extract was found to be reasonably useful in the treatment of *eczema* and no allergy or irritation was observed in treated patients (Jekayinfa et al., 1997).

A previous investigation of the antibacterial activity of the methanol *A. conyzoides* whole plant extract was

**Table 5.** Antibacterial and antifungal activities of plant extracts (MBC and MFC, µg/ml).

Extract	Microorganism								
	<i>C. a.</i>	<i>Es. c.</i>	<i>En. p.</i>	<i>K. p.</i>	<i>K. o.</i>	<i>P. m.</i>	<i>S. f.</i>	<i>S. a.</i>	<i>S. t.</i>
<b><i>A. wilkesiana</i></b>									
Aqueous	62.5	250	250	500	125	62.5	125	500	250
Methanol	125	500	500	250	250	125	500	250	500
<b><i>A. conyzoides</i></b>									
Aqueous	62.5	125	250	500	250	125	62.5	250	125
Methanol	125	250	250	125	250	250	125	250	250
<b><i>B. tholoniana</i></b>									
Aqueous	250	1000	500	250	500	500	500	125	1000
Methanol	1000	1000	500	500	500	500	250	125	250
<b><i>G. demeussi</i></b>									
Aqueous	1000	250	1000	500	250	62.5	250	250	125
<b><i>P. macrophylla</i></b>									
Aqueous	125	125	125	125	62.5	62.5	62.5	125	125
Methanol	62.5	125	62.5	250	62.5	62.5	125	125	250
<b><i>S. guineense</i></b>									
Aqueous	500	1000	250	1000	125	250	500	500	500
Methanol	250	1000	125	500	250	500	500	500	500
Norfloxacin	-	125	62.5	250	500	125	250	125	125
Tetracycline	-	15.62	15.61	7.80	7.80	3.90	1.95	1.95	0.97
Nystatin	0.25	-	-	-	-	-	-	-	-

compared to that of the leaves and flowers, and the results had indicated that the two last extracts were more effective than the first one against the tested bacteria such as *S. aureus*, *E. coli* and *P. aeruginosa* (Dayie et al., 2008). The essential oil of *A. conyzoides* leaves was also reported to have antibacterial activity against a large microorganisms and yeasts by diffusion method (Osho and Adetunji, 2011). In addition, it had been reported that wounds treated with the aqueous leaves extract in combination with honey and with solcosery ointment significantly accelerate wound healing process and the rates of wounds sterility compared to wound treated with honey alone (Mustafa et al., 2005.). Another study conducted by Dash and Murthy (2011) demonstrated that the methanol and aqueous extract from *A. conyzoides* leaves showed father rate of wound healing compared to

the effect of petroleum and chloroform extract from the same plant part. Among these later extracts, the chloroform extract was found to produce promising effect, but lower compared to that of the methanol and aqueous extracts, while the petroleum ether extract did not produce significant effect. In a previous study, a series of triterpene compounds were isolated from the leaves of *S. guineense* among which arjumolic acid and asiatic acid showed the most significant antibacterial activity against *E. coli*, *B. subtilis* and *S. sonnei* (Djoukeng et al., 2005).

Our results also indicated that the antibacterial and antifungal activities vary with the plant species, the plant part used and the nature of the extractive solvent (Malesh and Satish, 2008; Rios and Recio, 2005). Moreover, these reported results in sometime compared to those from previous screenings of other medicinal plants for the

same biological activities, showed good effect against Gram-positive strains while Gram-negative remained resistant (Vlietinck et al., 1995), (Rabe and van Staden, 1997). The observed biological activities in all selected plant extracts are related to the presence of some secondary metabolites such as terpenes, steroids, alkaloids, flavonoids, saponins and tannins found to be present in these crude extracts for which they are well known to possess these activities at different extents (Helberg et al., 1983; Ebi et al., 1997; Cowan, 1999; Mendonça Filho, 2006, Das, 2010).

## Conclusion

These selected plant extracts which are used for the treatment of several infections in traditional medicine demonstrate an appreciable *in vitro* antibacterial and antifungal, and at some extents bactericidal and fungicidal activities. Thus there is now a scientific validation for the use of the selected plant part extracts for the treating of infectious diseases in traditional medicine.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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*Full Length Research Paper*

## Antioxidant effects of curcumin against cadmium chloride-induced oxidative stress in the blood of rats

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Humans are exposed to a number of toxic elements in the environment. Cadmium, widely used in industry, is a great environmental health problem of both humans and animals. Effects of reactive oxygen species (ROS) generation have been postulated to be major contributors to cadmium-exposure related disease. The aim of this study was to investigate the effect of curcumin on oxidative stress in rats exposed to cadmium. Curcumin was administered orally (600 mg/kg body weight). After 24 days, significant increases in methemoglobin percentage (metHb%), superoxide dismutase (SOD), glutathione peroxidase (GPx) activity, malondialdehyde (MDA) concentration and hemolysis test were observed in cadmium exposed rats compared to control group ( $P < 0.05$ ), while GSH concentration showed insignificant change. Curcumin treatment of cadmium exposed rats significantly lowered metHb%, while significantly increased oxyhemoglobin percentage (HbO<sub>2</sub>%), compared to cadmium alone group ( $P < 0.05$ ). Also curcumin treatment significantly increased GPx activity of cadmium exposed rats as compared to cadmium alone group ( $P < 0.05$ ). Curcumin treatment of cadmium exposed rats lowered MDA concentration and hemolysis percentage by 10 and 9%, respectively. The findings of this study suggest that curcumin elevated the GPx activity of cadmium exposed rats and had ameliorative effect on lipid peroxidation and erythrocytes hemolysis. Moreover, the results of multi-component spectrophotometric analysis suggest that curcumin treatment of lead exposed rats lowered the levels of inactive metHb level and elevated the level of active HbO<sub>2</sub>. Curcumin may exert its protective actions against cadmium-induced hematotoxicity in rats possibly through its antioxidant mechanisms and may have future therapeutic relevance.

**Key words:** Cadmium, curcumin, oxidative stress, erythrocytes, hemolysis, Hb-derivatives, rats.

### INTRODUCTION

Cadmium (Cd) is an important industrial and environmental pollutant that currently ranks seventh on the Agency, for Toxic Substances and Disease Registry (ATSDR)/EPA list of hazardous substances (ATSDR

2003.). Cd will invariably be present in our society, either in useful products in the form of nickel-cadmium batteries, dyes, plastics, electrochemistry, paint pigments or in controlled wastes as a major source of pollution, in water

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and as a constituent of food material (Jarup et al., 1998; Ikeda et al., 2000). Cd is an omnipresent heavy metal which enters the biological systems from natural sources, such as volcanic emissions, weathering of rocks, mining processes as well as from industrial applications, agricultural practices and human usages. Cd emissions into the environment are normally continuous between the three main environmental compartments, air, water and soil. The majority of Cd exposure arises from ingestion of food substances due to uptake of Cd by plants from fertilizers, sewage, sludge, manure and atmospheric deposition (Anderson et al., 1988; Hotz et al., 1989; Lauwerys et al., 1991; Iwata et al., 1992; Bernard et al., 1992; Ikeda et al., 2000). Human uptake of Cd is mainly through cigarette smoking, food and water intake. In vegetarian diet, mushrooms, cacao powder, potatoes, fruits, wheat, grains, bran, sugarbeet fiber, carrot, dried seaweeds, etc., are the source of Cd intake. Similarly, in non-vegetarian diets shellfish, mussel, meat and fish are rich in cadmium. These Cd rich foods can greatly increase cadmium concentration in the human body (Friberg et al., 1985; Vahter et al., 1996; WHO, 2000). Therefore, Cd is a wide-spread environmental pollutant, characterized by its toxicity to various organs, including kidney, liver, lung, testis, brain, bone, blood system (Gunnarsson et al., 2003; WHO, 1992). The molecular mechanisms of its toxicity are not yet well defined. Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Manca et al., 1991; Shaikh et al., 1999). After the intake and resorption, Cd enters the blood where it binds to the red blood cell (RBC) membranes and plasma albumin (Bauman et al., 1993). In the blood and tissues, Cd stimulates the formation of metallothioneins (Simpkins et al., 1998) and reactive oxygen species (ROS), thus causing oxidative damage in RBCs and in various tissues, which result in a loss of membrane functions (Sarkar et al., 1995). Cd also induces the onset of anemia, decreases the RBC count, hemoglobin concentration and hematocrit value as well as producing reduced blood iron levels (Kostić et al., 1993). Moreover, a variety of accompanying changes in antioxidant defense enzymes were reported (Zikić et al., 1998; Kostić et al., 1993).

Antioxidants are the natural defense mechanism existing in our system and these are capable of scavenging the deleterious free radicals. A number of dietary antioxidant compounds have been shown to influence the membrane characteristics such as fluidity, stability and susceptibility to membrane oxidative damage (Halliwell and Gutteridge, 1990). Recently, a great deal of attention has focused on the protective biochemical functions of naturally occurring antioxidants in biological systems against toxic heavy metals. Thus, it is believed that antioxidant should be one of the important components

of an effective therapy of Cd poisoning. Curcumin, a yellow coloring ingredient of the spice turmeric obtained from the rhizomes of *Curcuma longa* Linn (Zingiberaceae), a perennial herb cultivated in upper Egypt and distributed mainly throughout the tropical and subtropical regions of the world. Curcumin represents a class of anti-inflammatory and antioxidants reported to be a potent inhibitor of ROS formation (Venkatesan et al., 2000; Biswas et al., 2005). Reddy and Lokesh (1994a) indicated that curcumin is a potent scavenger of a variety of ROS including superoxide anion radicals ( $O_2^{\cdot-}$ ) and hydroxyl radicals ( $\cdot OH$ ). Curcumin administration has been reported to prevent the arsenic, gentamicin and acetaminophen-induced oxidative stress in rats (Fatma et al., 2009; Farombi and Ektor, 2006; Cekmen et al., 2009). The protective effects of curcumin against chemically-induced hepatotoxicity are well documented and have been attributed to its intrinsic antioxidant properties (Nanji et al., 2003; Rukkumani et al., 2004). Curcumin, like many antioxidants, can be a "double-edge sword," whereby, in the test tube, carcinogenic and pro-oxidant effects may be seen in addition to anticancer and antioxidant effects (Kawanishi et al., 2005). However, a previous *in vivo* study reported that gavage administration of 200 or 600 mg/kg curcumin effectively suppressed diethylnitrosamine (DEN)-induced liver inflammation and hyperplasia in rats, as evidenced by histopathological examination (Chuang et al., 2000). The antioxidant effects of vitamin E and taurine against cadmium chloride-induced oxidative stress to erythrocytes and hematotoxicity in rats have been studied (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009). However, effects of curcumin, as a powerful antioxidant, on cadmium chloride-induced oxidative stress to erythrocytes and hematotoxicity in rats have not yet been studied. Therefore, the aim of this study was to investigate the antioxidant effects of curcumin against cadmium chloride-induced perturbations in oxidative biomarkers of blood such as levels of Hb-derivatives, plasma MDA level and GSH concentration, SOD and GPx activities in erythrocytes hemolysate and erythrocytes hemolysis test, in rats.

## MATERIALS AND METHODS

Eighteen male albino rats (age: 6 to 8 weeks and about 80 to 110 g body weight) were obtained from the animal house, National Research Center, Cairo, Egypt. All animals were treated in accordance to the principles of Laboratory Animal Facilities of World Health Organization, Geneva, Switzerland (2003). The animals were fed a standard pellet diet and had free access to water. The standard diet contained 50% wheat, 21% corn, 20% soybean, 8% concentrated proteins and 1% a mixture of salts, vitamins and dicalcium phosphate. The nutritional content was 5% fat, 21% protein, 55% nitrogen free extract and 4% fibre (w/w) with adequate minerals and vitamin contents. The rats were housed in stainless steel cages in a temperature-controlled room ( $25 \pm 2^\circ C$ ) with a 12 h light and 12 h dark exposure.

### Grouping of animals and treatment

The animals were randomly divided into three groups of 6 animals each, control, cadmium chloride alone, and cadmium chloride with curcumin. All groups were given a standard rat chow and water. Rats in cadmium alone and cadmium with curcumin groups were given treatments orally by gavage needle for 24 days. Rats of cadmium alone group were given daily 2 ml dose of a solution containing 10 mg/kg body weight of monohydrated cadmium chloride orally. While, rats of cadmium with curcumin group received a daily dose of a solution containing 10 mg/kg body weight of monohydrated cadmium chloride and 600 mg/kg body weight of curcumin dissolved in 2 ml of distilled water orally. The dose of curcumin used in this study was selected on the basis of the previous study (Chuang et al., 2000). Curcumin, an active component of turmeric (*C. longa* Linn) and a yellow coloured phenolic pigment yield from the rhizome of this tumeric, was purchased in a powder form from Elgabry Company for medicinal herbs, Giza, Egypt.

### Animal sacrifice and collection of samples

The experiments lasted for 24 days. At the end of the experimental period, blood samples were collected from all animals from the retro-orbital venous plexus. The blood samples were collected into heparinized tubes. The plasma obtained after centrifugation (3000 rpm for 10 min at 4°C) was used for MDA determination. Erythrocytes were washed three times in phosphate buffered saline (PBS) solution. Lysed erythrocytes were prepared by addition of four volumes of ice-cold distilled water. Cell membranes were removed by centrifugation at 8,500 rpm for 20 min, and the supernatant was used for the assay of GSH concentration and antioxidant enzymes activities. According to the antioxidant assays, appropriate phosphate buffers of pH 7 for GPx and pH 8.5 for SOD were added to the hemolysate samples; therefore, the antioxidant enzymes do not lose their activities.

### Biophysical assays

Levels of hemoglobin derivatives (sulfhemoglobin (SHb), methemoglobin (metHb), carboxyhemoglobin (HbCO), and oxyhemoglobin (HbO<sub>2</sub>)) in blood of rats were determined by the multicomponent spectrophotometric method described previously (Attia et al., 2011a). Percentages of erythrocytes hemolysis were determined according to the method of Attia et al. (2011b).

### Biochemical assays

For biochemical analysis specially manufactured kits were used. Reduced glutathione (GSH) concentration was determined spectrophotometrically by the method of Beutler et al. (1963). The method based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. Plasma malondialdehyde (MDA) concentration was determined spectrophotometrically by the method of Satoh (1978). Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resulting pink product can be measured at 534 nm. Superoxide dismutase (SOD) activity was determined spectrophotometrically by the method of Nishikimi et al. (1972). The principle of this assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The percent inhibition directly proportional to SOD activity was calculated,

depending on the increase in absorbance at 560 nm for control and sample, respectively. Gutathione peroxidase (GPx) activity was determined spectrophotometrically by the method of Paglia and Valentine (1967). The assay is an indirect measure of the activity of cellular GPx (c-GPx). Oxidized glutathione (GSSG), produced upon reduction of an organic peroxide by c-GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR). The oxidation of NADPH to NADP<sup>+</sup> is accompanied by a decrease in absorbance at 340 nm (A<sub>340</sub>) providing a spectrophotometric means for monitoring GPx enzyme activity. To assay c-GPx, erythrocytes hemolysate is added to a solution containing glutathione, glutathione reductase, and NADPH. The enzyme reaction is initiated by adding the substrate, hydrogen peroxide and the A<sub>340</sub> is recorded. The rate of decrease in the A<sub>340</sub> is directly proportional to the GPx activity in the sample.

### Statistical analysis

Data were presented as the mean ± standard error (SE) values. One way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Post Hoc and the least significant difference (LSD) tests using a statistical package program (SPSS version 14). P < 0.05 was considered as statistically significant.

## RESULTS

### Blood hemoglobin derivatives

Table 1 shows the levels of inactive hemoglobins (SHb, metHb, HbCO) and active HbO<sub>2</sub> in all groups. After 24 days, significant increase in metHb% was observed in cadmium-exposed rats, compared to the control group (P < 0.05), while cadmium treatment had no significant effects on SHb% and HbCO%. Curcumin treatment of cadmium exposed rats significantly lowered metHb%, while significantly increased HbO<sub>2</sub>%, compared to the cadmium alone group (P < 0.05).

### Plasma MDA concentration

Table 2 shows the concentration of MDA in plasma of all groups. After 24 days, significant increase in MDA concentration was observed in cadmium exposed rats as compared to control group (P < 0.005). Curcumin treatment of cadmium exposed rats lowered MDA concentration (P < 0.05, -10%) compared to cadmium alone group.

### Erythrocyte antioxidant enzyme activities and GSH concentration

Table 2 shows the concentration of GSH as well as the activities of SOD and GPx in erythrocytes of all groups. Superoxide dismutase (SOD) and GPx activity significantly increased (P < 0.05) in cadmium compared to the control group, while GSH concentration showed insignificant change. Curcumin treatment significantly increased

**Table 1.** Effects of cadmium chloride and curcumin on levels of inactive and active hemoglobins in blood of rats.

Parameter	Control	Cadmium chloride	Cadmium chloride + curcumin
SHb%	0.942 ± 0.241	0.993 ± 0.178	0.758 ± 0.127
metHb%	1.215 ± 0.112	1.680 ± 0.327 <sup>a</sup>	0.961 ± 0.402 <sup>b</sup>
HbCO%	3.203 ± 0.257	2.901 ± 0.270	3.121 ± 0.260
HbO <sub>2</sub> %	94.634 ± 0.159	94.425 ± 0.530	95.201 ± 0.345 <sup>b</sup>

Data are presented as mean ± standard error (SE). <sup>a</sup>Significantly different from control  $P < 0.05$ .  
<sup>b</sup>Significantly different from cadmium alone treatment group  $P < 0.05$ .

**Table 2.** Effects of cadmium chloride and curcumin on concentrations of plasma MDA, GSH, SOD and GPx activities in erythrocyte hemolysate in rats.

Parameter	Control	Cadmium chloride	Cadmium + curcumin
MDA (nmol/ml)	12.359 ± 0.957	17.844 ± 0.487 <sup>b</sup>	16.06 ± 1.275 <sup>a,c</sup>
GSH (mg/dl)	4.018 ± 0.301	4.246 ± 0.366	4.445 ± 0.391
SOD (U/g Hb)	6396.134 ± 873.252	9512.439 ± 703.613 <sup>a</sup>	10356.180 ± 954.806 <sup>b</sup>
GPx (mU/ml)	145.90 ± 5.978	170.757 ± 12.714 <sup>a</sup>	190.211 ± 7.971 <sup>b,c</sup>

Data are presented as mean ± standard error (SE). <sup>a</sup>Significantly different from control  $P < 0.05$ .  
<sup>b</sup>Significantly different from control-  $P < 0.005$ . <sup>c</sup> significantly different from cadmium alone treatment group  $P < 0.05$ .

increased GPx activity of cadmium exposed rats as compared to cadmium alone group ( $P < 0.05$ ), while it has no effect on SOD activity and GSH concentration. However, SOD and GPx activity of cadmium+curcumin group is significantly higher ( $P < 0.005$ ) than controls.

### Percentages of erythrocytes hemolysis

Figure 1 shows the hemolysis percent of erythrocytes in all groups. The hemolysis test indicates that intoxication by cadmium significantly increases the hemolytic effect ( $P < 0.05$ ), whereas after treatment with curcumin, it decreases by 9%.

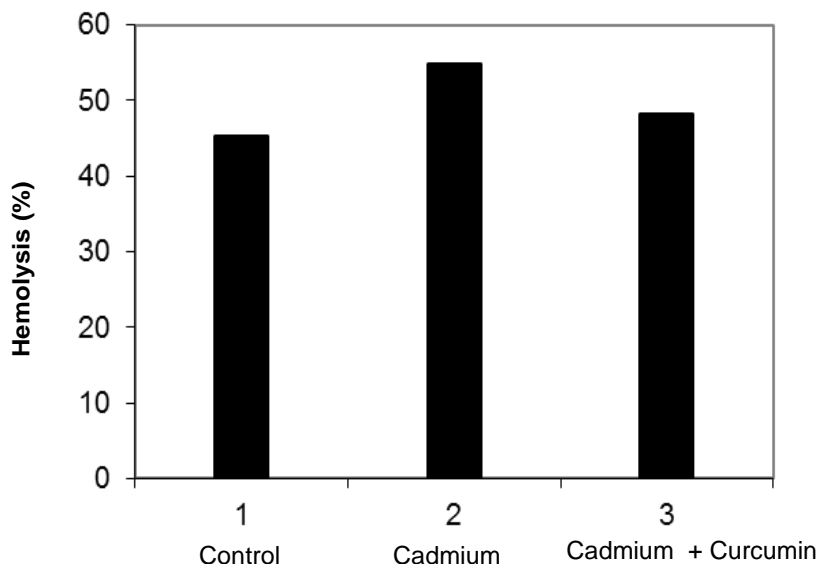
### DISCUSSION

Cd is a toxic metal that is widely used in different industries. The Agency for Toxic Substances and Disease Registry (ATSDR, 1989) in Atlanta, Georgia has listed Cd as a number 7 in its top 20 list of hazardous substances. It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions, because of its long retention in some tissues (Bagchi et al., 2000). The present results have clearly demonstrated the ability of Cd to induce oxidative stress in rat blood as evidenced by increased lipid peroxidation after 24 days of Cd treatment. This finding is in agreement with several reports demonstrating that Cd

induces oxidative stress in tissues by increasing lipid peroxidation and altering the antioxidant status in several tissues (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009; Onwuka et al., 2011; Tarasub et al., 2011, 2012; El-Sokkary et al., 2009).

This study showed that erythrocytes hemolysis in Cd treated animals is higher than controls consistent with a previous study (Kanter et al., 2009). This high rate of Cd-induced hemolysis decreases by 9% after curcumin treatment. Curcumin represents a class of antioxidants reported to be a potent inhibitor of ROS formation (Venkatesan et al., 2000; Biswas et al., 2005). Reddy and Lokesh (1994b) indicated that curcumin is a potent scavenger of a variety of ROS including superoxide anion radicals ( $O_2^{\cdot-}$ ) and hydroxyl radicals ( $\cdot OH$ ). This high antioxidant activity of curcumin can account for the decrease in lipid peroxidation and erythrocyte hemolysis after curcumin treatment of Cd-exposed rats, observed in this study.

The inactive components of Hb (SHb, metHb and HbCO) are unable to transport oxygen, while HbO<sub>2</sub> is the active Hb. When the iron atom is in the ferrous form, the protein is active and can bind oxygen reversibly. The oxidation to the ferric form (metHb) leads to an inactive protein. Methemoglobin is unable to carry oxygen. High oxidative stress in red blood cells of cadmium exposed animals can account for the increase in metHb% produced through HbO<sub>2</sub>-autoxidation reactions (Waltkins et al., 1985) and its improvement after treatment with curcumin can account for the decrease in metHb% and



**Figure 1.** Percentages of erythrocytes hemolysis in all groups. Hemolysis test was carried out after incubation of erythrocytes in phosphate buffered saline (PBS), pH 7.4, for 3 days at room temperature. <sup>a</sup>Significantly different from control P < 0.05, <sup>b</sup>Significantly different from cadmium alone treatment group P < 0.05.

increase in HbO<sub>2</sub>% observed in the present study.

Previous investigations showed that chronic treatment with Cd induced oxidative damage in erythrocytes of rats, causing destruction of red cell membranes and increased lipid peroxidation, as well as alteration of the antioxidant defense system, energy metabolism and appearance of anemia (Kostić et al., 1993; Kanter et al., 2005; Ognjanović et al., 2000; Zikić et al., 1997, 2001; Pavlovic et al., 2001).

The results obtained in our present study show that treatment with Cd induces an increase of the level of lipid peroxidation product, MDA, in the blood of rats, which were accompanied by increased formation of ROS (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009). As a consequence of enhanced lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis as well as marked marker disturbances of antioxidant defense system occurred (Hiruku and Kawanishi, 1996). Treatment with curcumin was effective in decreasing oxidative damage induced by Cd which resulted in markedly lower MDA concentration. Curcumin was capable of inhibiting formation of ROS which caused hemolysis, through its high antioxidant activity (Venkatesan et al., 2000; Biswas et al., 2005; Reddy and Lokesh, 1994a).

It is assumed that except of therapeutic intervention by using potent chelating agents capable to mobilize intracellularly bound Cd (Eybl et al., 1984; Jones and Cherian, 1990), curcumin as antioxidants may be important components of an effective Cd intoxication treatment. The inhibitory effect of turmeric on Cd induced lipid

peroxidation in blood was in parallel with Lalitha and Selvam (1999) who suggested that, curcumin provided a protection against lipid peroxidation and hemolysis of RBCs induced by H<sub>2</sub>O<sub>2</sub>.

In animals exposed to Cd, the activities of SOD and GPx in RBC were significantly increased (Table 2). These results are consistent with previous studies (Zikić et al., 2001; Ognjanović et al., 2003; Kanter et al., 2005, 2009). It is known that Cd induces the formation of superoxide anion radicals in erythrocytes and it is reasonable to expect an increased activity of SOD. Cd induced an increase in GPx activity which may be explained by their influence on hydrogen peroxide as substrate which is formed in the process of dismutation of superoxide anion radicals (Shaikh et al., 1999). The treatment with curcumin of Cd exposed rats increased GPx activity, indicating that this substance eliminates the toxic effects of Cd on the activity of this enzyme. At the same time, erythrocyte GSH concentration remains at the level of control values which confirm the protective role of curcumin. Moreover, curcumin treatment enhances the activity of SOD as compared to controls. The indirect antioxidant capacity of curcumin is defined by its ability to induce the expression of antioxidant enzymes such as SOD (Panchal et al., 2008) and GPx (Yarru et al., 2009). The antioxidant enzymes induced by curcumin are regulated by the nuclear factor erythroid-derived 2 (Nrf2) (Cuadrado et al., 2009; Rojo et al., 2012), which in turns is also activated by curcumin (Calabrese et al., 2008; Egger et al., 2008).

The antioxidant mechanism of curcumin was attributed

to its conjugated structure which includes two methoxylated phenols and an enol form of  $\beta$ -diketone. The structure showed a typical radical trapping ability as a chain breaking antioxidant (Masuda et al., 2001). Curcumin exhibit a differential antioxidant activity in several *in vitro* and *in vivo* models, for example, preventing lipid peroxidation in a variety of cells such as erythrocytes and rat liver microsomes, where peroxidation is induced by Fenton's reagent, as well as for metals and hydrogen peroxide ( $H_2O_2$ ) (Reddy and Aggarwal, 1994b). Furthermore, it has been reported that curcumin is a bifunctional antioxidant (Dinkova-Kostova and Talalay, 2008), because of its ability to react directly with reactive species and to induce an up-regulation of various cytoprotective and antioxidant proteins. Curcumin is able to scavenge superoxide anion ( $O_2^{\cdot-}$ ) (Ak and Gülçin, 2008; Sreejayan and Rao, 1996), hydroxyl radicals ( $\cdot OH$ ) (Barzegar and Moosavi-Movahedi, 2011), singlet oxygen (Das and Das, 2002), nitric oxide (Sreejayan, 1997; Sumanont et al., 2004), peroxytrite (Kim et al., 2003) and peroxy radicals ( $ROO\cdot$ ) (Barzegar and Moosavi-Movahedi, 2011). Together, these mechanisms might explain, at least in part, some of the cytoprotective effects of this compound. Features as the presence of phenolic groups in the structure of curcumin explains its ability to react with reactive oxygen species (ROS) and reactive nitrogen species (RNS) and might probably be one of the mechanisms through which curcumin treatment protects erythrocytes from oxidative damage.

It can be concluded from the presented results that cadmium induced oxidative damage in erythrocytes, leading to loss of membrane function by enhanced lipid peroxidation as well as alteration of the activity of antioxidant enzymes. Moreover, the results of multi-component spectrophotometric analysis showed an increase in the level of inactive methemoglobin (metHb). Curcumin expressed protective role against toxic influence of cadmium on all affected parameters in rats. Curcumin may exert its protective actions against cadmium-induced hematotoxicity in rats possibly through its antioxidant mechanisms. The results raise the possibility of curcumin being considered as one of the component of the regular diet of the people in the areas, where they may have chances of exposure to cadmium occupationally or environmentally.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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