African Journal of Biotechnology Vol. 10(56), pp. 12058-12063, 26 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1431
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Antimicrobial efficacy of *Rheum palmatum, Curcuma longa* and *Alpinia officinarum* extracts against some pathogenic microorganisms

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Accepted 12 August, 2011

The use and search for antibiotics and dietary supplements derived from plants have accelerated in recent years. Three plants, used traditionally as medicine and as food additive in Saudi Arabia, were collected and extracted with either methanol or n-butanol. The used plants were *Rheum palmatum, Curcuma longa* and *Alpinia officinarum*. The plant extracts were screened for their inhibitory effects on seven bacterial and five fungal genera using agar well diffusion method. It was shown that methanol extract was more effective as compared to n-butanol extracts. The minimum inhibitory concentrations (MIC) of the methanol extracts of the used plants ranged from 50 to 175 µg/ml. No toxicity was found using *Artimia salina* as test organism. Antitumor activity against Ehrlich ascites carcinoma was recorded only for *C. longa* extract.

Key words: Antimicrobial, antibiotic, *Rheum palmatum, Curcuma longa, Alpinia officinarum*, toxicity, minimum inhibitory concentrations, Ehrlich ascites carcinoma.

INTRODUCTION

Nowadays, there is a need to find naturally occurring substances with antimicrobial activity as an alternative to available antibiotics due to several serious problems such as growing drug resistance of bacteria or undesirable side effects of antibiotics (Ushimaru et al., 2007). Plants have been shown to be a rich source of antimicrobial agents, as they produce a wide variety of secondary compounds as natural protection against microbial attack (Urszula et al., 2010). Different plant extracts are employed for their antibacterial, antifungal and antiviral activities and it is know that more than 400,000 species of tropical flowering plants have antimicrobial activities (Odugbemi, 2006). Different plant parts are used for medical purposes including rhizomes, bulbs, leaves, roots, borks and peels (Anne-Catherine, 2007). *Alpinia officinarum, Curcuma longa* and *Rheum palmatum* have been used for a long time in Saudi Arabia as tradition medicinal plants but their antimicrobial activities were poorly discussed. *A. officinarum,* widely cultivated in South East of Asia, belongs to the family Zingeberaceae. The most active part of the plant is the rhizome which is characterized by dark reddish brown color and strong aromatic odor. Ethnomedical uses of this rhizome are found to be against rheumatism and children whooping cough (Kirtikar and Basu, 2001; Srividya et al., 2010). *C. longa* is a medicinal plant that is botanically related to family Zingiberaceae (Chattopadhyay et al., 2004). It possesses properties like antioxidant, anti-inflammatory, antiplatelet and antimicrobial effects in addition to cholesterol lowering activity (Shaguflanaz et al., 2010). *Rheum genus* belongs to the family Polygonaceae and it is popularly used for many therapeutic purposes. The extract was more active against reference strains of Gram negative and positive bacteria *(*Urszula et al., 2010).

The continuous evolutions of bacterial resistance to currently available antibiotics have necessitated the search for novel and effective antimicrobial compounds

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from medicinal plants. In this study, the rhizome extracts of *A. officinarum, C. longa* and *R. palmatum* which were used in alternative medicine and as food additive were screened for their antimicrobial, toxicity and antitumor activities.

MATERIALS AND METHODS

Source of microorganisms

Pathogenic bacteria and fungi used in this study as test organisms were obtained from the culture collection of Dr. R. Bonally, Laboratoire de Biochimie Microbienne, Fac. De Pharmacie, Nancy, France.

Collection of plant materials

Dried roots and rhizomes of *A. officinarum, C. longa* and *R. palmatum* were collected from Jeddah market, Saudi Arabia during summer 2009 and identified at the Biology Department, Faculty of Sciences, KAU, Jeddah, Saudi Arabia.

Preparation of the plant extracts

Plant materials were washed individually with distilled water and oven-dried for 6 h at 160°C. Each plant was grinded into fine powder and about 10 g of each plant was extracted by 100 ml of either 95% methanol or n-butanol (1:10 w/v) for 24 h. The slurries obtained were left in clean sterile glass containers and was shaken vigorously to allow for proper extraction. The slurries were filtered using a sterile filter paper and each extract obtained was concentrated to dryness at 50°C under vacuum, dissolved in dimethyl sulphoxide (DMSO) and stored at 4°C until used (Aly and Bafeel, 2008).

Antimicrobial screening tests

The sensitivity of the pathogenic bacteria and fungi, used as test organisms, to the plant extracts was carried out using agar well diffusion method as described by Holder and Boyce (1994). Preculture of each test organism was prepared using either nutrient broth medium for bacteria or Sabouraud medium for fungi. A sterile pipette was used to add 1 ml of the preculture containing 4×10^6 CFU/ml of bacteria or 6×10^4 of fungi to each Petri dish containing 15 ml of already prepared Muller Hinton agar medium. Wells of 7 mm in diameter were made in the seeded agar using sterile cork borer and about 100 µl of the extract were added to each well. The resulting inhibition zones were measured in millimeters. DMSO was used as negative control and ampicillin and amphotericin B were used as standard antibacterial and antifungal agents, respectively (Agwa et al., 2000).

Minimal inhibitory concentration (MIC)

The MIC was determined using the methods described by Chand et al. (1994) and modified by Aly (1997). Each well of a 96 well ELISA tray was filled with 175 µl of an exponentially growing culture (10^6 ~ 10^7CFU ml^{-1}). To each well, 20 µl solution of each concentration of the tested extract, or the appropriate solvent as control, was added. The ELISA trays were incubated for 40 min before 5 µl of a 0.2% (w/v) solution of Fluorescein diacetate (FDA) in acetone was added. Incubation was continued for 90 min and the resulting green color from the hydrolysis of FDA was measured at 490 nm (MR7000 automatic ELISA tray reader) and blanked against control wells containing microbial cultures only.

Toxicity (brine shrimp lethality) test of plant extracts

Brine shrimp lethality bioassay was carried out to investigate the toxicity of the plant extracts. 50 mg of *Artemia salina* (Leach) eggs were added to a hatching chamber containing seawater (75 ml). The hatching chamber was kept under an inflorescent lamp for 48 h for the eggs to hatch into shrimp larvae. 50 mg of each dried plant extract was separately dissolved in 2 ml of methanol and different dilution were made including 400, 300, 200 and 100 µg/ml in small vials and each dilution was tested in triplicate. 10 larvae of *A. salina* were added to each vial. The final volume of solution in each vial was adjusted to 5 ml with seawater immediately after adding the shrimps. Percentage of cell inhibitory of the brine shrimps obtained for plant extracts were recorded (Adoum, 2009).

Antitumor activity of the plant extract

Ehrlich ascites carcinoma cell line was supplied by the National Cancer institute of Tanta, Egypt. The cells were grown in RPMI 1640 medium (Sigma, USA) with 10% fetal calf serum (FCS) (Gibco, USA) at 37°C under a humidified atmosphere consisting of 95% air and 5% CO_2 for 48 h. Ehrlich ascites carcinoma cells were treated with different doses of the plant extract for 24 h. Cells were centrifuged for 2 min at 1500 g and were counted after removing the supernatant using hemacytometer and trypan blue (Sigma, USA) in normal saline (1:1 v/v). The percentage of cell viability was assessed to determine the lethal dose by which 50% of cells are killed (LD_{50}).

Statistical analysis

Each experiment has three replicates and three determinations were conducted. Means of variable and standard deviation were recorded. Student t- test was carried out to detect any significant differences between the results of control and the treated samples.

RESULTS AND DISCUSSION

The investigation of plants for bioactive secondary metabolites has become inevitable due to significant correlation between their uses in traditional medicine and the observed bioeffects of their extracts (Fatope, 1995). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Crown, 1999).

R. palmatum, *C. longa* and *A. officinarum* were collected, extracted with either methanol or butanol and their antimicrobial activities were recorded against different bacteria, yeasts and fungi that were used as test organisms. In the case of using bacteria (Table 1), the

Table 1. The antibacterial activities (diameter of inhibition zone, mm) of methanol and n-butanol extracts of the three tested plants against some pathogenic bacteria.

Used bacteria	*Rheum palmatum*		*Curcuma longa*		*Alpinia officinarum*	
	Methanol extract	Butanol extract	Methanol extract	Butanol extract	Methanol extract	Butanol extract
Escherichia coli	20±2.1	20±0.2	27±4.1	22±0.2	24±0.12	22±0.0
Pseudomonas aeuroginosa	21±3.0	20±0.2	26±9.1	24±0.2	16±0.35	14±0.2
Shigella dysenteriae	*20±*2.2	20±0.2	25±3.	21±0.2	18±0.54	14±0.31
Klebsiella pneumoniae	22±6.1	18±0.2	23±11.0	20±0.2	14±0.44	12±0.1
Bacillus subtilis	17±3.2	14±0.2	27±6.2	14±0.2	10±0.60	10±0.0
Staphylococcus aureus	16±2.4	14±0.2	26±4.4	16±0.2	10±0.31	10±0.1
Micrococcus roseus	15±2.2	12±0.2	25±2.3	16±0.2	10±0.18	11±0.1
Bacterial index**	18.78*	16.8*	25.5*	19.0*	14.5*	13.2*

**Bacterial index: Total activities against bacteria divided by the number of the tested bacteria; *, significant results at p≤0.05 as compared to the control (DMSO).

diameter of inhibition zone ranged from 25 to 27 mm with mean antibacterial index of 25 mm and from 14 to 24 mm with mean index of 19 mm for methanol and butanol *Curcuma* extracts, respectively. Both extracts of *R. palmatum* showed moderate antibacterial activity, the diameter of inhibition zone ranged from 15 to 20 mm for methanolic extract and from 12 to 20 mm for the extract of n-butanol. The lowest antibacterial activity was obtained by *A. officinarum*, the diameter of inhibition zone ranged from 10 to 24 mm for methanolic extract and from 10 to 22 mm for butanolic extract. Based on these results, the methanolic extract was more effective as compared to n-butanol extract. This result is correlated with the previous studies that reported that methanol was a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents such as water and ethanol (Ahmad et al., 1998). This conflict can explain that the better extraction of antimicrobial compounds from various medicinal plants may require different solvents.

The antifungal activity of the methanolic extracts of the studied plants is shown in Table 2. The methanolic extract of *C. longa* (antifungal index 18.3 mm) was the most active against the tested fungi followed by the extract *A. officinarum* and *R. palmatum*, respectively. From the previous results, it is clear that the antibacterial activity of the methanolic extract of *C. longa* was greater than the activity of *R. palmatum* which was greater than the activity of *A. officinarum.* The antifungal activity of *C. longa* was greater than the activity of *A. officinarum* which was greater than the activity of *R. palmatum.* Similarly, the results obtained by Huang et al. (2008) showed that the extract of *Alpinia* showed moderate to potent antibacterial activity against *Staphylococcus aureus*, α and β-hemolytic *Streptococcus* and *Streptococcus pneumonia* due to excellent inhibition of beta-ketoacyl-ACP reductase system in bacteria. Khattak et al. (2005) reported that *C. longa* and *A. officinarum* extracts were found to possess good antifungal activities against *Trichophyton longifusus* (65 and 60% fungal inhibition, respectively). The results of Rambir et al. (2002) confirmed that *C. longa* rhizome extracts showed antibacterial activity against *Sraphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa and Salmonnella typhimurium*. Similarly, Shagufta et al. (2010) confirmed that among all the bacterial strains, *Bacillus subtilis* was the most sensitive to *C. longa* extracts. Whatever, Kosikowska et al. (2010) found an excellent inhibitory effect against *Staphylococcus* spp. using *Rheum* extract, with MIC ranging from 125 to 250 µg/ml. They suggested that this plant, which is often used in the European cuisine to improve flavor, might be also important and useful as an alternative or auxiliary medicine remedy in the treatment of uncomplicated superficial infections caused especially by clinically important staphylococci, potentially pathogenic *S. aureus* or opportunistic *S. epidermidis*.

MICs of the three selected plant extracts were determined using flurocin diacetate method and compared with that of ampicillin and amphotericin B. Ampicillin is a β-lactam antibiotic that has been used extensively to treat bacterial infections. However, amphotericin B is a polyenic antifungal agent used to control pathogenic yeast and fungi. The MIC of ampicillin against different tested bacteria ranged from 2 to 10 µg/ml. Furthermore, MICs were 50 to 100, 50 and 75 to 100 µg/ml for *R. palmatum*, *C. longa* and *A. officinarum*, respectively (Table 3).

The antifungal activities of the three tested plants were determined against yeasts and fungi (Table 4) and the MIC ranged from 100 to 150 µg/ml. Aly (1997) found that MIC of amphotericin B for pathogenic yeasts was 5 µg/ml. It can be concluded that, MICs for the three selected plants were greater than that obtained for

Table 2. The antifungal activities (diameter of inhibition zone, mm) of the methanol extracts of the three plants against some pathogenic yeasts and fungi.

Tested fungi	*Rheum palmatum*	*Curcuma longa*	*Alpinia officinarum*
Candida albicans	10±0.1	24±0.11	18±0.11
Candida trobicals	11±0.2	23±0.12	17±0.12
Creptococcus neoformans	10±0.0	22±0.51	17±0.21
Alterneria solani	17±0.0	10±0.23	14±0.31
Fusarium oxosporium	19±0.0	10±0.54	10±0.52
Aspergillus niger	24±0.44	11±0.14	18±0.72
Antifungal index**	15.1*	18.8*	17.8*

**Antifungal index: Total activities against bacteria divided by the number of the tested bacteria. *significant results at $p<0.05$ as compared to the control (DMSO).

Table 3. MIC expressed in µg/ml of methanol extracts of the rhizomes of the three selected plants against different bacteria as compared to a standard antibiotic (ampicillin).

Bacteria	*Rheum palmatum*	*Curcuma longa*	*Alpinia officinarum*	Ampicillin
Escherichia coli	50±12.1	50±5.1	75±11.2	2±0.3
Pseudomonas aeuroginosa	50±3.0	50±6.2	75±15.2	2±0.1
Shigella dysenteriae	50±5.2	50±7.2	75±13.2	2±0.1
Klebsiella pneumoniae	50±4.1	50±1.0	75±8.0	5±0.1
Bacillus subtilis	100±911	50±9.2	75±11.2	5±0.1
Staphylococcus aureus	75±7.4	50±4.3	75±12.4	5±0.6
Micrococcus roseus	75±10.2	50±13.3	75±10.2	5±0.1

Table 4. MIC expressed in µg/ml of methanol extracts of the rhizomes of three selected plants against different fungi as compared to a standard antifungal agent (Amphotericin B).

Fungi	*Rheum palmatum*	*Curcuma longa*	*Alpinia officinarum*	Amphotericin B
Candida albicans	150±0.1	100±13.3	100±0.11	5±0.1
Candida trobicals	150±0.2	100±13.3	100±0.12	20±0.1
Creptococcus neoformans	150±0.0	100±13.3	100±0.21	5±0.1
Alterneria solani	100±0.0	100±13.3	150±0.31	10±0.1
Fusarium oxosporium	100±0.0	100±13.3	150±0.52	10±0.1
Aspergillus niger	100±0.44	100±13.3	100±0.72	10±0.1

ampicillin or amphotericin B. Further studies are needed to determine the active compound(s) in such plant extracts as well as its formulation to be applicable as alternative materials to be used in the treatment of pathogenic bacteria, yeast and fungi. Therefore, such results are of a significant value that confirms the therapeutic potency of some plants used in traditional medicine. It should form a good basis for further phytochemical and pharmaco-logical investigation. Useful phytochemical antimicrobial agents are polyphenols (simple phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins); terpenoids; essential oils; alkaloids; lectins; polypeptides and other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisms vary and depend on these compounds (Aly and Bafeel, 2008). Their mechanism of actions may include enzyme inhibition by the oxidized compounds that act as a source of stable free radical and often lead to inactivation of the protein and loss of function (Aly and Bafeel, 2010). Plant extract may contain active component that have the ability to complex with extracellular and soluble proteins

Table 5. Toxicity and antitumor activity of the three tested plant extracts.

Tested plant	Toxicity against *A. salina* (% of cell inhibition)					Antitumor activity (LC_{50}, µg/ml)
	Control (0 µg/ml)	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	
Curcuma longa	0	8	10	14	27**	100*
Rheum palmatum	0	11	14	20	24**	>400
Alpinia officinarum	0	17	20	22	27**	>400

** No toxicity (% of cell inhibition less than 50%) was recorded; *significant result at $p<0.05$ as compared to the control (DMSO).

of the microbial cell and/or to complex with bacterial cell walls and disrupt microbial membranes (Ali, 1999). Some extracts may have ability to intercalate with DNA, form ion channels in the microbial membrane, and have competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Cowan, 1999; Bokhari, 2009).

In the course of searching for antitumor agents, our results show that the methanolic extract of *C. longa* exhibited excellent antitumor activity against Ehrlish ascites carcinoma cell line (Table 5) with LC_{50} of 100 µg/ml; however, no activity was found using the extracts of *R. palmatum* or *A. officinarum* up to 400 µg/ml. Similarly, many authors reported that *C. longa* has been shown to possess anticancer activity (Chuang et al., 2000; Skrzypezac-Jankun et al., 2000; Bala et al., 2010) and antitumor activities (Unnikrishnan and Rao, 1995). Furthermore, laboratory animal model studies have suggested that *Curcuma* may play an important role in inhibiting the process of carcinogenesis and was effective in inducing apoptosis or programmed cell death in human myeloid leukemia cells (HL-60) in addition to *Curcuma* containing active compounds act as antitumor agent (Kuo et al., 1996). Two active compounds named ar-turmerone and β-atlantone were identified and the exposure of human myeloid leukemia HL-60 cells to clinical achievable concentrations of ar-turmerone or β-atlantone produced internucleoside DNA fragmentation of approximately 200 base pair multiples and the morphological changes characteristic of cells undergoing apoptosis or programmed cell death. These findings suggest that these agents may exert their antitumor activity, in part, through induction of apoptosis (Cui et al., 2006).

Toxicity studies of several local plant extracts on insects and fish must be carried out before been applied on animal (Aly and Bafeel, 2010). Therefore, screening of plant extracts for toxicity effects have been carried out but never exhausted (Adoum et al., 1997; Araújo and Leon, 2001). Biological testing has played an important role in toxicity studies of plant extracts and there are numerous bioassay studies to detect the toxicity of the plant extracts. In our study, no toxicity was recorded for the extracts of *A. officinarum, R. palmatum* and *C. longa* up to 400 µg/ml using *A. salina* as the test organisms. Some plant extracts including *Mentha arvensis, Eugenia caryophyllus* and *Decaspermum momtanum* exhibited 100% mortality, whereas extract of *Cymbopogon citratus* exhibited about 30% mortality at the same concentration (Sukari, 1992). Contrarily, the ethanolic extracts of *C. longa* and *A. galanga* exhibited excellent phytotoxic activity (100%) against *Lemna minor* at higher concentration (Khattak et al., 2005), while in the brine shrimp, lethality bioassay were found to be toxic with LD_{50} of 33 and 109 µg/ml, respectively.

In conclusion, the crude rhizome extracts of *R. palmatum, C. longa* and *A. officinarum* that exhibited good antibacterial and antifungal activities with no toxicity should be evaluated further in-depth to isolate the active component(s) to be used in alternative medicine.

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