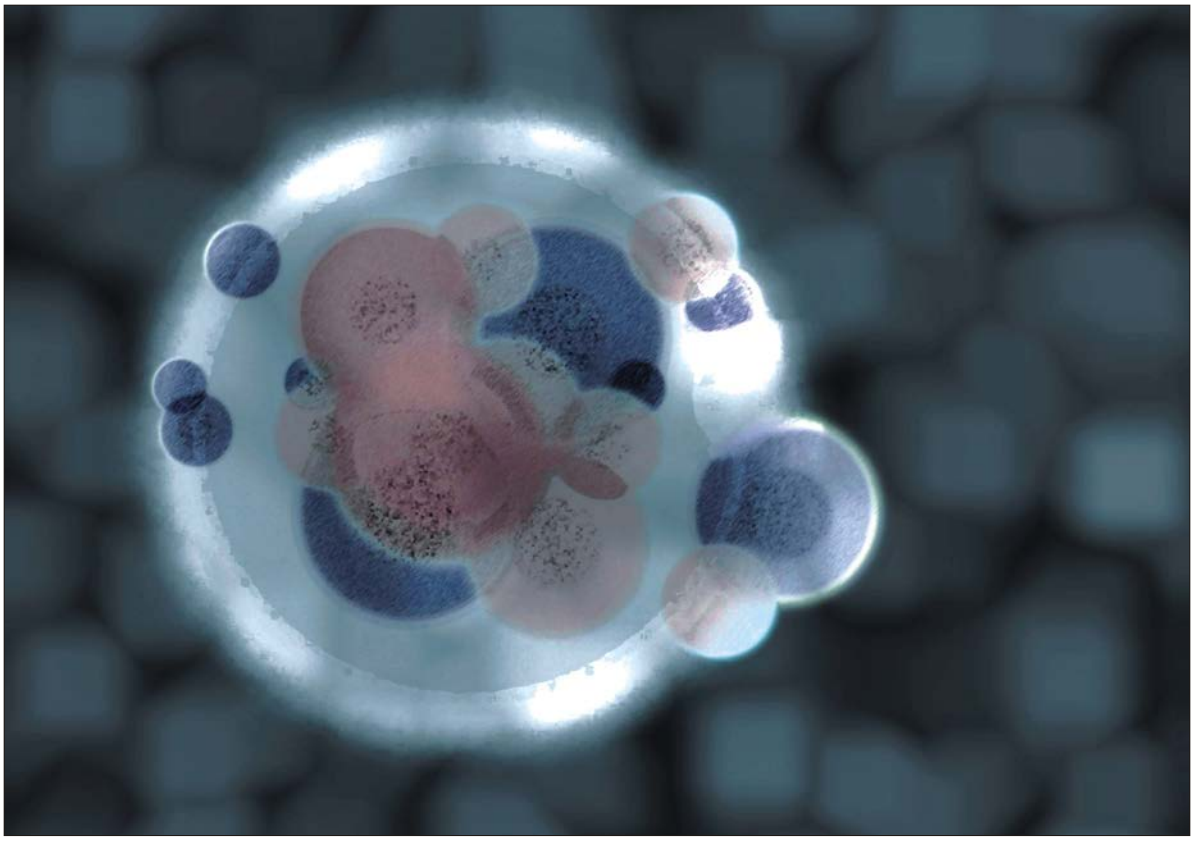

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Comparative Study on the *in vitro* Antibacterial Efficacy of Aqueous and Methanolic Extracts of *Quercus infectoria* Gall's Against *Cellulosimicrobium cellulans*

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Abstract: The *in vitro* antibacterial efficacy of aqueous and methanolic extract of *Quercus infectoria* Olivier (Fagaceae) galls was tested against *Cellulosimicrobium cellulans* using extract concentration ranging from 0.25 to 4 mg mL^{-1}. Both types of extract showed significant inhibition of *C. cellulans* growth with strong correlation between extract concentrations and degrees of antibacterial activity for concentrations ranging from 0.5 to 4 mg mL^{-1}. Although, slight reduction of average diameter of inhibition zones after 24 h of incubation for aqueous extract (0.96±0.148 cm) compared to methanolic extract (1.00±0.182 cm), both extracts still attained the MIC value beginning at a concentration of 0.5 mg mL^{-1} but established higher concentration for the MBC at 2 mg mL^{-1}. The antibacterial activity of methanolic extract was also significantly affected by the temperature with an optimum inhibition zone being obtained at 30°C (1.38±0.05 cm) and this was reduced to approximately 20% at temperatures of above 50°C.

Key words: Antibacterial, aqueous extract, *Cellulosimicrobium cellulans*, methanolic extract, *Quercus infectoria*

INTRODUCTION

Investigations of traditionally used plants for biologically active extracts had been well-documented. Recent studies have revealed that medicinal plants from various parts of the world could be rich sources of antibacterial and antimicrobial activities (Voravuthikunchai and Kitpipit, 2005; Cowan, 1999). Antibiotic-resistant bacteria (Voravuthikunchai *et al*., 2006), stimulation of toxin production (Cowan, 1999) and the recent upturn in consumer mistrust of synthetic additives have pushed the search for natural compounds from plants to replace antibiotics or artificial antimicrobials. Even though certain plants have been demonstrated to have effects against pathogenic bacteria, the majority of them have not yet been investigated for their antibacterial activities.

In this research, *Quercus infectoria* Olivier (Fagaceae) was studied in order to investigate its antibacterial properties. *Quercus infectoria* is a round-shaped abnormal growth found arising on young branches of the oak tree as a result of attack by the gall-wasp Adleria gallae-tinctoria (Samuelsson, 1999). Oak gall consists of gland (camata, fruit in cupola), hoof (trillo; sharp and stubby points covered with cupola) and cup (cupula; outside surface of oak gall). Research has shown that *Q. infectoria* is rich in bioactive compounds such as tannin (Haghi and Safaei, 2004), vitamins A and C, calcium, iron, fiber, protein and carbohydrates (Jalalpure *et al*., 2002) and has the ability to be an antimicrobial (Everest and Ozturk, 2005), antibacterial (Hamid *et al*., 2005) and antifungal agent (Yamunarani *et al*., 2005).

Cellulosimicrobium cellulans (previously identified as *Oerskovia xanthineolytica* or *Brevibacterium fermentans* or *Arthrobacter luteus*), a gram-positive bacterium belonging to the order Actinomycetales (Schumann *et al*., 2001) was selected to determine the antibacterial activity of *Q. infectoria*. *Cellulosimicrobium cellulans* is relatively a virulent and rarely associated with infections in humans (Rowlinson *et al*., 2006). However, it has been associated with the presence of foreign bodies and is generally found in immunocompromised patients (Kaur *et al*., 2004). Early case reports described meningitis and sepsis in infants and children due to infection by *C. cellulans* which at in the late 60s was known as *B. fermentans* (Tenover, 2001; Kailath *et al*., 1988). A few years later, other reports related *C. cellulans* to

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endocarditis, pyonephrosis, endophthalmitis, pneumonia, meningitis, parenteral nutrition-related septicemia, catheter-related septicemia and peritonitis (Maguire *et al*., 1996).

In this study, the screening of the antibacterial activities of methanolic and aqueous extracts of the galls of *Q. infectoria* against *C. cellulans* by the determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) are described. This study also investigated the effects of different solvents and temperatures on these activities.

MATERIALS AND METHODS

The experiment: This experiment was conducted at Plant Systematics and Microbial Laboratory, Biology Department, Universiti Putra Malaysia, Malaysia from July to September 2007.

Plant material: *Quercus infectoria* Olivier (Fagaceae) was purchased from the local herbal shop. The oak galls were washed with water, then surface sterilized with 10% sodium hypochlorite solution before they were rinsed with sterile distilled water and air dried at 30°C. Then the samples were crushed into pieces and grinded into powder using a grinder before being sieved to get only a fine powder.

Preparation of plant extracts: Fifty grams of *Q. infectoria* fine powder were soaked in 125 mL of 80% (w/v) methanol or homogenized in sterile distilled water for preparing the methanolic and aqueous extracts, respectively. This mixture was then stirred for 24 h at 30°C and filtered using Whatman No. 1 filter paper. Lastly, the filtrate was evaporated by using rotary evaporator (40-60°C) until fully dried.

Preparation of test extract solutions: A series of different *Q. infectoria* extract concentrations (0.25, 0.50, 0.75, 1, 2, 3 and 4 mg mL^{-1}) were prepared by dissolving a known weight of the plant extract in 5% (v/v) dimethyl sulphoxide (DMSO) which acted as a solvent.

Preparation of microorganism culture: *Cellulosimicrobium cellulans* ATCC 21606 was obtained from the Plant Systematics and Microbial Laboratory, Biology Department, Universiti Putra Malaysia and maintained on Nutrient Agar slant. Preparation of the test strain culture was carried out by isolating a single colony of *C. cellulans* from the nutrient agar and transferred into 250 mL Nutrient Broth before being shaken at 180 rpm for 24 h.

Antibacterial assays: Agar well diffusion assay: The agar well diffusion was prepared by adding 1×105 cfu mL^{-1} *C. cellulans* culture into melt nutrient agar and homogenized slowly for a few seconds. The mixture was then poured into a petri dish and allowed to solidify prior to the preparation of 0.6 cm diameter wells made by using a sterilized cork borer. Twenty microliter of *Q. infectoria* extract solution in different concentration were transferred into each well and allowed to set. All the plates were incubated at 37°C and the diameters of the inhibition zone surrounding each well were measured to the nearest millimeter at every 12 h internal. Sets of 5 replicates were used for each type of extract.

Determination of the Minimum Inhibitory Concentration (MIC): This process was conducted using microtiter plates and the same series of *Q. infectoria* extract concentrations as previously used in the antibacterial assays. Twenty microliter of *Q. infectoria* extract were mixed into 80 μL sterile Nutrient Broth before 1×105 cfu mL^{-1} *C. cellulans* culture was added. The microtiter plates were incubated at 37°C for 24 h and the lowest *Q. infectoria* extract concentration that did not show any growth of *C. cellulans* after microscopic evaluations was determined as being the MIC.

Determination of the Minimum Bacterialcidal Concentration (MBC): All the microtiter wells used in the MIC determination which did not show any growth of *C. cellulans* after the incubation period were subcultures onto fresh Nutrient Agar plates and incubated further for 24 h at 37°C. The least concentration with no *C. cellulans* growth was considered as being the MBC value.

Effects of temperature on antibacterial activity: The MBC concentration for *Q. infectoria* extract solution from the antibacterial assay experiment was used in this study. The six different temperatures tested in this experiment were 10, 30, 50, 70, 90 and 100°C, respectively. Twenty microliter of *Q. infectoria* extract solution was heated to temperature required before being allowed to cool to room temperature and dispensed into the well made on a Nutrient Agar. Five replicates of each concentration were prepared and all the plates were incubated at 37°C for 120 h before the diameters of the inhibition zones were measured.

Statistical analysis: The results obtained were expressed as Mean±SEM. The data were analyzed using the Tukey test at the 5% significance level.

RESULTS AND DISCUSSION

The overall inhibitory concentration value of the methanolic and aqueous extracts from the galls of *Q. infectoria* against *C. cellulans* are shown in Table 1; with methanol 80% (v/v) as a negative control treatment with no inhibitory effect on the bacteria tested. Inhibition started to appear for extract for both solvents at 0.5 mg mL^{-1}. However this inhibition zone can only be sustained for 24 h before it became cloudy and the bacteria colony started to grow. The outcome of this antibacterial assay was supported by the result of the MIC determination (Table 1) which showed that no *C. cellulans* could be seen under microscopic observation. In spite of this, values of MBC were much higher for both solvents being at 2 mg mL^{-1} *Q. infectoria* extract. This means that at 0.5 mg mL^{-1} concentration, aqueous and methanolic extracts of *Q. infectoria* could only be act as bacteriostatic agents rather than as bactericidal for *C. cellulans*. At this concentration, the bioactive compound was unable to eliminate *C. cellulans* or to sustain the activity for a long period thus allowing the bacteria to grow. Two possible explanations for this bacteriostatic effect are (i) the bioactive compound in the extract was not adequate to cause significant mortality to the bacteria (Basri and Fan, 2005) (ii) the sensitivity of the bioactive compound toward a certain type of solvent might cause or enhance the rate of deactivation or degradation (Matu and Staden, 2003).

After 24 h incubation, all concentrations except for 0.25 mg mL^{-1} for both solvents had shown drastically increases in the diameter of the inhibition zones with averages of 0.96±0.148 and 1.00±0.182 cm for the aqueous and methanolic extracts, respectively. This might be directly caused by immature bacteria being less resistant to antibacterial activities at this stage (Beukinga *et al*., 2004). However, slow drops in the inhibition zones diameters were observed after 48 h and after 120 h the sizes were reduced by about 3% for the aqueous and 2.2% for the methanolic extracts. The unsustainable antibacterial activities might due to the enzyme starting to degrade and the bacteria becoming much more dominant (Beukinga *et al*., 2004). Nonetheless, there were still strong correlations between extract concentrations and the diameters of the inhibition zones at this stage with correlation coefficients of 0.735 and 0.790 for the aqueous and the methanolic extracts, respectively. The concentration of the extract played an important role in the antibacterial activity in that a higher antibacterial activity would be obtained with a concentrated extract (de Boer *et al*., 2004; Sawangjaroen *et al*., 2004).

The fact that *C. cellulans* was categorized as a gram-positive bacteria (Schumann *et al*., 2001) has some contribution towards the effectiveness of *Q. infectoria* extract as a bacteriostatic or bactericidal agent. Generally plant extracts are much more active against gram-positive bacteria than against gram-negative bacteria (Lin *et al*., 1999; Cimanga *et al*., 2002) and this was demonstrated by the positive effects of several plants extracts on other gram-positive bacteria such as *S. epidermidis*, *Bacillus subtilis* (Fatima *et al*., 2001) and *Pseudomonas aeruginosa* (Nimri *et al*., 1999).

Table 1: Antibacterial activity of the *Q. infectoria* extract prepared using different solvents against *C. cellulans*

		Diameter of inhibition zone (cm±SD)*							
		Incubation period (h)							
Extract	Concentration (mg mL^{-1})	0	24	48	72	96	120	MIC**	MBC***
Methanolic	Control	0.60±0.00^{a}	0.60±0.00^{e}	0.60±0.00^{d}	0.60±0.00^{e}	0.60±0.00^{e}	0.60±0.00^{e}	-	ND
	0.25	0.60±0.00^{a}	0.60±0.00^{e}	0.60±0.00^{d}	0.60±0.00^{e}	0.60±0.00^{e}	0.60±0.00^{e}	-	ND
	0.50	0.60±0.00^{a}	0.77±0.03^{b}	0.78±0.03^{c}	0.77±0.03^{b}	0.75±0.04^{b}	0.79±0.03^{b}	±	ND
	0.75	0.60±0.00^{a}	0.91±0.05^{b}	0.88±0.04^{c}	0.79±0.03^{b}	0.81±0.04^{b}	0.84±0.03^{b}	+	√
	1.00	0.60±0.00^{a}	0.88±0.04^{b}	0.84±0.06^{c}	0.88±0.03^{b}	0.87±0.05^{b}	0.89±0.05^{b}	+	√
	2.00	0.60±0.00^{a}	1.04±0.05^{a}	1.02±0.02^{b}	0.99±0.04^{b}	0.98±0.04^{b}	0.91±0.06^{a}	+	X
	3.00	0.60±0.00^{a}	1.16±0.05^{a}	1.08±0.04^{b}	1.08±0.05^{b}	1.09±0.03^{b}	1.13±0.05^{a}	+	X
	4.00	0.60±0.00^{a}	1.26±0.06^{a}	1.25±0.05^{a}	1.20±0.06^{a}	1.19±0.05^{a}	1.23±0.06^{a}	+	X
Aqueous	Control	0.60±0.00^{a}	0.60±0.00^{d}	0.60±0.00^{d}	0.60±0.00^{d}	0.60±0.00^{e}	0.60±0.00^{e}	-	ND
	0.25	0.60±0.00^{a}	0.60±0.00^{d}	0.60±0.00^{d}	0.60±0.00^{d}	0.60±0.00^{e}	0.60±0.00^{e}	-	ND
	0.50	0.60±0.00^{a}	0.79±0.02^{c}	0.75±0.04^{c}	0.75±0.02^{c}	0.78±0.02^{b}	0.76±0.01^{b}	±	√
	0.75	0.60±0.00^{a}	0.85±0.05^{c}	0.79±0.04^{b}	0.77±0.03^{c}	0.80±0.02^{b}	0.82±0.01^{b}	±	√
	1.00	0.60±0.00^{a}	0.85±0.05^{c}	0.82±0.02^{b}	0.82±0.04^{c}	0.81±0.06^{b}	0.81±0.05^{b}	+	√
	2.00	0.60±0.00^{a}	0.98±0.05^{b}	0.93±0.06^{b}	0.82±0.04^{b}	0.93±0.04^{b}	0.95±0.03^{b}	+	X
	3.00	0.60±0.00^{a}	1.09±0.02^{b}	1.06±0.05^{b}	1.04±0.04^{a}	1.02±0.05^{a}	1.05±0.05^{b}	+	X
	4.00	0.60±0.00^{a}	1.21±0.05^{a}	1.17±0.08^{a}	1.12±0.06^{a}	1.19±0.08^{a}	1.17±0.07^{a}	+	X

Means in each column with same superscript letter are not significantly different amongst themselves when Tukey tests were used at 5% significance level.
* Diameter of the inhibition zone was included 0.6 cm of the well diameter and expressed as the mean±SD; (N = 5).
** Minimum Inhibition Concentration: (-) no inhibition zone observed; (±) cloudy zone at the later stage of incubation; (++) inhibition zone observed.
*** Minimum Bactericidal Concentration: ND: Not determined due to negative result in MIC; √: Presence of bacteria growth; X: No bacteria growth observed

The type of solvent used in the extract preparation also greatly influenced the bioactive compound extraction (Pinelo *et al*., 2005). Due to the difference in the degree of polarity between aqueous and methanol, a difference in antibacterial activity was expected. Although the means of the inhibition zones diameter for all concentrations after 120 h, incubation were larger for the methanolic when compared to the aqueous extract; analysis of variance (ANOVA; p = 0.05) showed no significant difference in size between these two types of solvents. The similarity in the antimicrobial activity of both extracts suggested that these extracts may have high total tannin contents as tannin is a major compound in *Q. infectoria* (Jalalpure *et al*., 2002) which is soluble in water, alcohol and acetone (Basri and Fan 2005). Tannin is a form of phenolic acid (Chung *et al*., 1998) and alcoholic solvents have been commonly employed to extract phenolics from natural sources even though alcoholic solvents are not highly selective for phenols because it is able to yield high quantities of total extract compared to other types of solvent (Spigno *et al*., 2006). This explained why the methanolic extract showed higher extraction capability compared to the aqueous extract and similar outcomes had been reported in comparisons of extraction capabilities between acetone and aqueous to extract bioactive compounds from *Euclea natalensis* (Lall and Meyer, 2000). The intermediate polarity solvents such as methylene dichloride, tetrahydrofuran, ethyl acetate and acetone were able to extract much higher quantities of plant compounds than polar (aqueous and methanol) or non-polar solvents such as hexane (Eloff *et al*., 2005). However, the use of alcohol and water mixture gave better phenolic constituents extraction than other solvent systems (Yilmaz and Toledo, 2006).

Two milligrams per milliliters of methanolic extract was selected to be studied further on the effects of temperature based on the results obtained from the antibacterial assay done previously. The largest inhibition was shown at 30°C, followed by 50 and 10°C, while almost similar size of inhibition zone was obtained for temperatures 70, 90 and 100°C (Table 2). The treatment at 30°C did preserve the antibacterial activity with the highest diameter of inhibition zone (1.38±0.05 cm) compared to the other temperatures. This was in accordance with earlier reports that most plant extracts released considerable heat and their effects were most optimum at this temperature (28-30°C) (Baysale *et al*., 2003). Thus, although the time and temperature of extraction are important parameters to be optimized in order to minimize the energy cost of the process and to enhance both the solubility of the solute and the diffusion coefficient (Eloff *et al*., 2005), they may also cause certain phenolic compounds to be denatured (Pinelo *et al*., 2005).

Table 2: Diameter of growth inhibition zone for *C. cellulans* after 120 h incubation with 2 mg mL^{-1} *Q. infectoria* methanolic extract heated to different temperatures

Temperature (°C)	Diameter of inhibition zone (cm±SD)*
10	1.25±0.04^{b}
30	1.38±0.05^{a}
50	1.36±0.05^{a}
70	1.11±0.01^{c}
90	1.18±0.03^{c}
100	1.17±0.03^{c}

Means in each column with same superscript letter(s) are not significantly different amongst themselves when Tukey tests were used at 5% significance level.*Diameter of the inhibition zone was included 0.6 cm of the well diameter and expressed as the mean±SD; (N = 5)

In conclusion, this study had demonstrated the ability of *Q. infectoria* extract to act as a bactericidal agent. However, this is much dependent on the concentration applied, the temperature and type of solvent used in the extraction process. The potential of *Q. infectoria* extract in pharmacology as an antibacterial agent is enormous, but further biochemical analysis is required to prove this.

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REFERENCES

Basri, D.F. and S.H. Fan, 2005. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J. Pharmacol., 37 (1): 26-69.

Baysale, M., M.K. Yalinkilic, M. Colak and O. Goktas, 2003. Combustion properties of calabrian pine (*Pinus brutia* Ten.) Wood treated with vegetable tanning extracts and boron compounds. Turk. J. Agric. For., 27 (4): 245-252.

Beukinga, I., H. Rodriguez-Villalobos, A. Deplano, F. Jacobs and M.J. Struelens, 2004. Management of long-term catheter-related *Brevibacterium bacteremia*. Clin. Microbiol. Infect., 10 (5): 465B467.

Chung, K.T., T.Y. Wong, C.I. Wei, Y.W. Huang and Y. Lin, 1998. Tannins and human health: A review. Crit. Rev. Food Sci. Nutr., 38 (6): 421B464.

Cimanga, K., K. Kambu, L. Tona, S. Apers, T. De Bruyne, N. Hermans, J. Tott´e, L. Pieters and A.J. Vlietinck, 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Ethnopharmacol., 79 (2): 213-220.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12 (4): 564-582.

de Boer, H.J., A. Kool, A. Broberg, W.R. Mziray, I. Hedberg and J.J. Levenfors, 2004. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol., 96 (3): 462-469.

Eloff, J.N., J.O. Famakin and D.R.P. Katerere, 2005. *Combretum woodii* (Combretaceae) leaf extracts have high activity against Gram-negative and Gram-positive bacteria. Afr. J. Biotechnol., 4 (10): 1161-1166.

Everest, A. and E. Ozturk, 2005. Focusing on the ethnobotanical uses of plants in Mersin and Adana provinces (Turkey). J. Ethnobiol. Ethnomed., 1 (1): 1-6.

Fatima, S., A.H.A. Farooqi, R. Kumar, T.R.S. Kumar and S.P.S. Khanuja, 2001. Antibacterial activity possessed by medicinal plants used in tooth powders. J. Med. Aromatic Plant Sci., 22 (2): 187-189.

Haghi, G. and A. Safaei, 2004. Identification and determination of polyphenols and tannin in the galls and in the extract of *Quercus infectoria*. Iran. J. Pharm. Res., 3 (2): 85-86.

Hamid, H., G. Kaur, S.T. Abdullah, M. Ali, M. Athar and M.S. Alam, 2005. Two New Compounds from the Galls of *Quercus infectoria* with Nitric Oxide and Superoxide Inhibiting Ability. Pharm. Biol., 43 (4): 317-323.

Jalalpure, S.S., M.B. Patil and K.R. Alagawadi, 2002. Wound healing activity of the galls of *Quercus infectoria* Olivier. J. Nat. Remed., 2 (1-2): 54-58.

Kailath, E.J., E. Goldstein and F.H. Wegner, 1988. Case report: Meningitis caused by *Oerskovia xanthineolytica*. Am. J. Med. Sci., 295 (3): 216-217.

Kaur, G., H. Hamid, A. Ali, M.S. Alam and M. Athar, 2004. Anti-inflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. J. Ethnopharmacol., 90 (2-3): 285-292.

Lall, N. and J.J.M. Meyer, 2000. Antibacterial activity of water and acetone extract of the root of *Euclea natalensis*. J. Etnopharmacol., 72 (2): 313-316.

Lin, J., A.R. Opaku, M. Geheeb-Keller, A.D. Hutchings, S.E. Terblanche and A.K. Jäger, 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J. Ethnopharmacol., 68 (1-3): 267-274.

Maguire, J.D., M.C. McCarthy and C.F. Decker, 1996. *Oerskovia xanthineolytica* bacteremia in an immunocompromised host. Clin. Infect. Dis., 22 (3): 554-556.

Matu, E.N. and J. Staden, 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J. Ethnopharmacol., 87 (1): 35-41.

Nimri, L.F., M.M. Meqdam and A. Alkofahi, 1999. Antibacterial activity of Jordanian medicinal plants. Pharm. Biol., 37 (3): 196-201.

Pinelo, M., P.D. Fabbro, L. Marzocco, M.J. Nunez and M.C. Vicoli, 2005. Optimization of continuous phenol extraction from *Vitis vinifera* by products. J. Food Chem., 92 (1): 109-117.

Rowlinson, M.C., D.A. Bruckner, C. Hinnebusch, K. Nielsen and J.G. Deville, 2006. Clearance of *Cellulosimicrobium cellulans* bacteremia in a child without central venous catheter removal. J. Clin. Microbiol., 44 (7): 2650-2654.

Samuelsson, G., 1999. Drugs of Natural Origin. Stockhholm. Swedish Pharmaceutical Press, pp: 551.

Sawangjaroen, N., K. Sawangjaroen and P. Poonpanang, 2004. Effects of Piper longum fruit, Piper sarmentosum root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. J. Ethnopharmacol., 91 (3): 357-360.

Schumann, P., N. Weiss and E. Stackebrandt, 2001. Reclassification of *Cellulomonas cellulans Cellulosimicrobium cellulans*. Int. J. Syst. Evol. Microbiol., 51 (3): 1007-1010.

Spigno, G., L. Tramelli and D.M. Faveri, 2006. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J. Food Eng., 81 (1): 200-208.

Tenover, F.C., 2001. Development and spread of bacterial resistance to antimicrobial agents: An overview. Clin. Infect. Dis., 33 (3): 108-115.

Voravuthikunchai, S.P. and L. Kitpipit, 2005. Activity of medicinal plant extracts against hospital strains of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect., 11 (6): 510-512.

Voravuthikunchai, S.P., S. Limsuwan, O. Supasol and S. Subhadhirasakul, 2006. Antibacterial activity of extracts from family Zingiberaceae against foodborne pathogens. J. Food Safety, 26 (4): 325-334.

Yamunarani, K., R. Jaganathan, R. Bhaskaran, P. Govindaraju and R. Velazhahan, 2005. *In vitro* Antifungal Activity of a 29-kDa Glycoprotein Purified from the Galls of *Quercus infectoria*. Acta Phytopath. Entomol. Hungarica, 40 (1-2): 43-54.

Yilmaz, Y. and R.T. Toledo, 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. J. Food Composit. Anal., 19 (1): 41-44.