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ORIGINAL ARTICLE

Chemical composition and antibacterial properties of the essential oils and crude extracts of *Merremia borneensis*

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2-Methyl-2-nitropropane;
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Antibacterial activity

Abstract The hydro distilled essential leaves and stems oils of *Merremia borneensis* were analysed by GC–MS. Sixty-nine compounds representing 96.81% and 89.89% of the leaves and stems oils, respectively, were identified, of which chloromethyl propanoate (3.29% and 3.54%), methylcyclopropanemethanol (1.29% and 1.03%), oxirane (1.41% and 1.05%), 1-penten-3-ol (1.33% and 1.12%), 1-(2-propenyloxy)-heptane (3.44% and 2.98%), camphene (4.11% and 3.65%), l-octen-3-ol (1.56% and 1.08%), α-pinene (2.98% and 2.12%), β-pinene (2.19% and 1.93%), 2-methyl-2-nitropropane (11.91% and 10.51%), bis(1,1-dimethylethyl)-diazene (1.25% and 1.71%), *p*-cymene (2.23% and 2.11%), limonene (1.28% and 1.11%), neopentane (12.02% and 11.95%), cyclopropyl methyl carbinol (2.19% and 1.99%), *cis*-2-octenal (1.29% and 1.13%), 4-undecanone (4.11% and 3.99%), menthone (1.99% and 1.73%), isomenthone (1.01% and 0.93%), methylchavicol (1.57% and 2.22%), dodecane (1.01% and 0.72%), eugenol (3.12% and 3.09%), β-elemene (1.99% and 1.89%), methyleugenol (1.42% and 1.13%), β-carryophyllene (1.12% and 1.05%), α-humulene (6.54% and 6.32%), tridecane (1.16% and 1.08%) were the major compounds. Thus, different types of monoterpenes and sesquiterpenes were the predominant portions of the oils. Essential oils and methanol extract of *M. borneensis* and the derived fractions of hexane, chloroform, and ethyl acetate were tested for antibacterial activity, which was determined by disc diffusion and minimum inhibitory concentration (MIC) determination methods. The oils, methanol extract and derived fractions of methanol extract did not display any potential of antibacterial activity against the tested 10 phytopathogenic bacterial such as *Enterobacter cloacae*, *Staphylococcus aureus*, *Escherichia coli*,

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Bacillus cereus, *Salmonella typhimurium*, *Salmonella biafra*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Vibrio parahaemolyticus*, in the range of 0% and minimum inhibitory concentration ranging from 25 to 100 μg/ml.

1. Introduction

Plants have great potential sources for producing new drugs of benefit to mankind. There are many approaches in the search for new biologically active principles in higher plants (Abramowize, 1990). Many efforts have been scientifically expended to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One such resource is folk medicine and systematic screening of these traditional herbs may result in the discovery of novel effective compounds (Ahmad et al., 1998; Aswal et al., 1996; Bauer et al., 1966).

Antibacterial properties of different parts of plant like roots, stems, leaves, flowers, fruit and seeds have been well documented for some of the medicinal plants for the past two decades (Aswal et al., 1996; Mitscher et al., 1987; Mourey and Canillac, 2002; Olah et al., 2003). Most of the medicinal and aromatic plants and essences are rich sources in antibacterial compounds which can be an alternative to combat bacterial diseases (Bauer et al., 1966; Benson, 1990; Fong, 1973; Fransworth and Loub, 1983; Janovska et al., 2003; Laven et al., 1979; Nelson, 2007). In recent years antimicrobial properties of Bangladeshi medicinal plants have been increasingly reported (Nelson, 2007; Samy et al., 1998; Schumutterer, 1990).

Chemical bactericides are known to be highly effective to control the postharvest diseases in various vegetables and fruits. Due to the health concerns associated with exposure risks such as health and environmental hazards, residue persistence, and development of tolerance they are not able to consider as long-term solutions (Lingk, 1991; Radja Commare et al., 2002). For the search of synthetic chemical bacteriocidal alternatives, the increasing recognition and importance of bacterial infections and the difficulties encountered in their treatment have stimulated. Recently, researchers have been very much interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics (Hunter and Reeves, 2002). Essential oils are made up of many different types of volatile compounds and have been shown to possess antimicrobial and antibacterial properties (Karmen et al., 2003; Ahmet et al., 2005). Essential oils and organic plant extracts are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses (Sawamura, 2000; Ormancey et al., 2001). So, essential oils and organic plant extracts are one of the most promising groups of natural compounds for the development of safer antibacterial agents.

Merremia borneensis is applied as a medicinal plant because of the diuretic, antifungal and bacteriostatic properties of its leaves (Prieto et al., 1999). Most of the scientific and academic papers dealing with this subject refer these effects to the content of potassium, inositol and lipophilic flavones in *M. borneensis* leaves (Schut and Zwaving, 1993; Schneider and Tan, 1973). In addition to the above-mentioned components, saponins, sterols, polyphenols, rosmarinic acid and ursolic acid and essential oil have been also detected (Stecher, 1976; Tezuka et al., 2000; Hossain et al., 2006; Guillen and Manzanos, 1998; Haznedaroglu et al., 2001; Jovanovic et al., 2005). The leaves are suitable to be used as wrapper to the famous fermented rice or fermented tapioca known in Malaysia as 'Tapai'. The medicinal plant creeps well and is very productive in shady areas as well as open areas and are known to blanket a wholesome tree or on any objects that it chooses to make its habitat. The stem contains latex that is highly sticky and the flowers are white in color. The Bilaran leaves, according to natives in Sarawak, Malaysia, are used to relieve breast cancer (Prieto et al., 1999). In some cases, the essential oil is the reason for diuretic effects of plant drugs, which has not yet been described in detail. Based on preliminary analyses, however, there is no report available in the literature on the detailed analyses of essential oil of *M. borneensis* and its antibacterial property.

Therefore, the aim of this present study is (a) to examine the chemical composition of the essential oils isolated from the leaves and stems of *M. borneensis* by GC–MS; and (b) to evaluate the anti-bacterial activity of essential oils and methanolic extract of *M. borneensis* and its derived fractions of hexane, chloroform and ethyl acetate against certain important phytopathogens causing severe diseases in the plants.

2. Materials and methods

2.1. Plant material

The leaves and stems of *M. borneensis* were collected from the campus area at University Malaysia Sabah in Malaysia, in November 2010 and initially identified by morphological features and data base present in the library, School of Biology, University Malaysia Sabah.

2.2. Sample collection

The fresh green leaves and stems of *M. borneensis* were collected from the campus of Universiti Malaysia Sabah, Malaysia. The leaves of this plant were harvested during the month of September 2010. The leaves and stems sample were collected at 2:00 pm–3:00 pm on September 2, 2010 and packed in polyethylene bags and stored at 4 °C until required. The plant samples were initially identified by morphological features and data base present in the library, School of Biology, University Malaysia Sabah, Malaysia. About 50 g of leaves were ground using a grinder (Blender 80115) for 20 s. The unfermented *M. borneensis* leaves and stems were kept in the oven at 40 °C and put in a desiccator for at least 24 h prior to analysis.

2.3. Isolation of the essential oil

The air-dried plant material (100 g) was subjected to hydro distillation for 3 h using a Clevenger type apparatus. The essential oil from leaves and stems samples were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4 °C until further analysis.

2.4. Preparation of crude extracts

The small pieces of leaves were homogenised in a grinder for 3 min to 30–40 mesh size. The air-dried leaves were pulverized into powdered form. The dried leaves powder (50 g) was extracted three times with 70% ethanol (3 × 200 ml) at room temperature and combined. The combined crude extracts were evaporated by a vacuum rotary evaporator (Buchi Labortech AG, model 1, R-215). The ethanol extract was (7.3 g) diluted by water and extracted successively with hexane, chloroform, ethyl acetate and butanol to give hexane (1.97 g), chloroform (0.93 g), ethyl acetate (0.78 g) and butanol (0.391 g) and residual ethanol fractions (0.58 g), respectively. The extract was filtered using Whatmann No. 41 filter paper to obtain particle free extract. The residue was reextracted twice by solvent and filtered. The extracts were pooled and then concentrated and dried under vacuum pressure. The same extraction procedure was followed for the other solvents such as hexane, ethyl acetate, chloroform and butanol for antioxidant fractions (Jovanovic et al., 2005; Kim and Shin, 2004; Mourey and Canillac, 2002) and the extracts were used to explore their total flavonoids and other biochemical screening. Solvents (analytical grade) for extraction were obtained from E-Merck.

2.5. GC–MS analysis

The GC–MS analysis of the essential oil from leaves and stems were performed using a Perkin Elmer GC–MS (Model Perkin Elmer Clarus 500, USA) equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 μm). For gas chromatography–mass spectroscopic detection, an electron ionization system with ionization energy of 70 eV was used. Inert helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Mass transfer line and Injector temperature were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 μl was manually injected in the splitless mode. The relative percentage of the essential oil constituents was expressed as percentage by peak area normalization.

Identification of chemical compounds of the essential oil was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with those of standards (Mainlab, Replib and Tutorial data of GC–MS systems) and, whenever possible, by co-injection with authentic compounds (Adam, 2001).

2.6. Microorganisms

The bacterial strain cultures were obtained from the Biotechnology Research Institute (IPB), Universiti Malaysia Sabah, Malaysia. Cultures of each bacterial species were maintained on nutrient agar plate. The 10 bacterial species used in the experiment were *Enterobacter cloacae*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Salmonella biafra*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Vibrio parahaemolyticus*.

2.7. Preparation of solvent extractions

The dry leaves powder of plant materials (25 g) were filled separately in the thimble and successively extracted with 150 ml each of methanol, ethanol, ethyl acetate and chloroform using a Soxhlet extractor for 72 h. All the extracts were concentrated using rotary evaporator. After complete solvent evaporation, each of these solvent extracts was weighed and preserved at 4 °C in airtight bottles until further use. 0.5 g of each dry crude extract was dissolved in 5 ml of respective solvents, which were used as the test extracts for antimicrobial activity assay.

2.7.1. Determination of antibacterial activity of essential oils and crude extracts

Antibacterial activity of aqueous extract and solvent extracts; methanol, ethanol, ethyl acetate and chloroform was determined by disc diffusion method on nutrient agar medium (Anonymous, 1996). Whatmann filter discs (7.5 mm diameter) were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 53 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 100 μl each of all aqueous and solvent extracts was placed in the discs made in inoculated plates. The treatments also included 100 μl of solvents served as control and chloromphenicol as a standard control. Plates were incubated for 24 h at 37 °C and zone of inhibition if any around the wells was measured in mm (millimeter). All treatment consists of three replicates and repeated at twice. The determination of minimum inhibitory concentration (MIC) as the lowest concentration of *M. borneensis* plant extracts inhibiting the growth of the organism, was determined based on the readings.

2.7.2. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of essential oils and crude methanol extract and its derived fractions were determined by a twofold dilution method against *E. cloacae*, *S. aureus*, *E. coli*, *B. cereus*, *S. typhimurium*, *S. biafra*, *K. pneumoniae*, *V. cholerae* and *V. parahaemolyticus* (Murray et al., 1995; Oumzil et al., 2002; Pattnaik et al., 1997). The essential oil and crude samples were dissolved in methanol according to their respective known weights. The mother solutions were serially diluted with methanol and were added to disc diffusion method on nutrient agar medium to final concentrations of 25, 50 and 100 μl, respectively. The minimum inhibition concentration (MIC) at which no visible growth was observed was defined as the MIC, which was expressed in mm.

3. Results

3.1. Chemical composition of essential oil

The essential oils analysis by using GC–MS had led to the identification of 69 different organic compounds, representing

Table 1 Percentage composition of the volatile leaves and stem oils of *Merremia borneensis*.

RI[a]	Compounds	Leaves (%)	Stems (%)
780	Hexanal	0.87	0.78
826	*trans*-2-Hexanal	0.91	0.72
843	*cis*-3-Hexen-l-ol	0.43	0.68
856	Hexan-1-ol	0.15	0.05
861	Chloromethyl propanoate	3.29	3.54
878	Methylcyclopropanemethanol	1.29	1.03
884	Hexan-1-ol	0.09	0.02
885	Pentanal	0.23	0.19
887	Oxirane	1.41	1.05
888	Heptenal	0.91	0.43
889	1-Penten-3-ol	1.33	1.12
893	1-(2-propenyloxy)-heptane	3.44	2.98
894	2-Pentanone	0.75	0.33
895	Camphene	4.11	3.65
896	l-Octen-3-ol	1.56	1.08
897	α-Pinene	2.98	2.12
899	β-Pinene	2.19	1.93
901	2-Methyl-2-nitropropane	11.91	10.51
917	Diazene, bis(1,1-dimethylethyl)-	1.25	1.71
926	Ethanedioic acid, bis(1-methylpropyl) ester	0.54	0.31
938	*p*-Cymene	2.23	2.11
955	1,8-Cineol	0.49	0.36
978	Limonene	1.28	1.11
987	Neopentane	12.02	11.95
991	3-Pentanone	0.12	0.09
1001	Cyclopropyl methyl carbinol	2.19	1.99
1003	Borinic acid, diethyl-	0.41	0.29
1005	Pentane, 2,2,4,4-tetramethyl-	0.09	0.05
1028	*cis*-2-Octenal	1.29	1.13
1029	Diborane	0.21	0.19
1034	4-Undecanone	4.11	3.99
1044	Carbonic acid, allyl butyl ester	0.71	0.23
1054	Linalool	0.44	0.41
1078	Perillen	0.12	0.15
1098	Camphor	0.97	0.67
1100	σ-Terpineol	0.51	0.59
1103	Menthone	1.99	1.73
1148	Isomenthone	1.01	0.94
1178	Methylchavicol	1.57	2.22
1184	Borneol	0.21	0.17
1189	Decanal	0.78	0.67
1190	Dodecane	1.01	0.72
1205	Carvone	0.77	0.54
1235	*trans*-Anethol	0.11	0.09
1278	Safranal	0.18	0.13
1279	Tridecan	0.55	0.29
1298	γ-Elemene	0.31	0.29
13.27	α-Cubebene	0.91	0.79
13.44	Damascenone	0.72	0.42
13.69	α-Copaene	0.61	0.38
13.82	Eugenol	3.12	3.09
13.93	β-Elemene	1.99	1.89
14.70	Methyleugenol	1.42	1.13
15.08	*cis*-Caryophyllene	0.15	0.18
15.49	β-Carryophyllene	1.12	1.05
15.78	α-Humulene	6.54	6.32
15.92	β-Ionone	0.23	0.21
16.01	Germacrene D	0.09	0.04
16.32	α-Muuiolene	0.41	0.33
16.72	σ-Cadinene	0.21	0.19
16.99	Germacrene B	0.11	0.23
17.39	Dehydroionone	0.39	0.31
17.44	2-Nonanone	0.79	0.54
17.89	Tridecane	1.16	1.08
17.91	Octadecane	0.88	0.89
18.11	Decane	0.53	0.56
18.25	Undecane, 2,2-dimethyl-	0.48	0.28
18.72	Decane, 2,2-dimethyl-	0.14	0.13
18.88	*n*-Butyl ether	0.25	0.28
Total		96.81	89.89

[a] Retention index relative to *n*-alkanes on VF-5 capillary column.

96.81% and 89.89% of the total oils from leaves and stems samples, respectively. The identified chemical compounds are listed in Table 1 according to their elution order on a VF-5 capillary column. The essential oil contains a complex mixture consisting of mainly oxygenated mono and sesquiterpene hydrocarbons. The major organic compounds detected in the leaves and stems oils, respectively, were chloromethyl propanoate (3.29% and 3.54%), methylcyclopropanemethanol (1.29% and 1.03%), oxirane (1.41% and 1.05%), 1-penten-3-ol (1.33% and 1.12%), 1-(2-propenyloxy)-heptane (3.44% and 2.98%), camphene (4.11% and 3.65%), l-octen-3-ol (1.56% and 1.08%), α-pinene (2.98% and 2.12%), β-pinene (2.19% and 1.93%), 2-methyl-2-nitropropane (11.91% and 10.51%), bis(1,1-dimethylethyl)-diazene (1.25% and 1.71%), *p*-cymene (2.23% and 2.11%), limonene (1.28% and 1.11%), neopentane (12.02% and 11.95%), cyclopropyl methyl carbinol (2.19% and 1.99%), *cis*-2-octenal (1.29% and 1.13%), 4-undecanone (4.11% and 3.99%), menthone (1.99% and 1.73%), isomenthone (1.01% and 0.93%), methylchavicol (1.57% and 2.22%), dodecane (1.01% and 0.72%), eugenol (3.12% and 3.09%), β-elemene (1.99% and 1.89%), methyleugenol (1.42% and 1.13%), β-carryophyllene (1.12% and 1.05%), α-humulene (6.54% and 6.32%), tridecane (1.16% and 1.08%) (Table 1). Mono- and sesquiterpene hydrocarbons were the characteristic constituents of the oils of *M. borneensis*. 1,8-Cineol, borneol, camphor, eugenol, carbinol and σ-terpineol were also found to be the minor components of *M. borneensis* leaves and stems oils in the present study.

3.2. Antibacterial activity of essential oils and crude extracts

The leaves and stems oils of *M. borneensis* did not show any antibacterial activity against all the tested bacterial strains at the three concentrations of 25, 50 and 100 μg/ml, as shown in Table 2. Also, the crude methanol extract from the leaves samples and its derived hexane, chloroform and ethyl acetate fractions did not show disc diffusion method on nutrient agar medium against all phytopathogens. According to the experimental result given in Table 3, methanol extract and other organic extracts of *M. borneensis* also did not show any anti bacterial effect may be due to low concentration of essential oils and all organic extracts. So our next study will focus on different concentrations of essential oils and all organic extracts and check the activity of all bacterial strains.

Table 2 Antibacterial activity of essential oil of leaves and stems 5 µl (25, 50 and 100 µg/ml) of *Merremia borneensis*.

Bacterial strains	Leaves essential oil				Stems essential oil			
	Growth inhibition[a]			MIC (µg/ml)	Growth Inhibition[a]			MIC (µg/ml)
	25 µl	50 µl	100 µl		25 µl	50 µl	100 µl	
Enterobacter cloacae	–	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–	–
Escherichia coli	–	–	–	–	–	–	–	–
Bacillus cereus	–	–	–	–	–	–	–	–
Salmonella typhimurium	–	–	–	–	–	–	–	–
Salmonella biafra	–	–	–	–	–	–	–	–
Klebsiella pneumoniae	–	–	–	–	–	–	–	–
Vibrio cholerae	–	–	–	–	–	–	–	–
Vibrio parahaemolyticus	–	–	–	–	–	–	–	–

–: No detection of antibacterial activity.
[a] Values are represented as the mean ± S.D. of three experiments.

Table 3 Antibacterial activity of methanol extract and its derived fractions 10 µl (25, 50 and 100 µg/ml) of *Merremia borneensis*.

Bacterial strains	Growth inhibition[a]			
	CME	HAF	EAF	CHF
Enterobacter cloacae	–	–	–	–
Staphylococcus aureus	–	–	–	–
Escherichia coli	–	–	–	–
Bacillus cereus	–	–	–	–
Salmonella typhimurium	–	–	–	–
Salmonella biafra	–	–	–	–
Klebsiella pneumoniae	–	–	–	–
Vibrio cholerae	–	–	–	–
Vibrio parahaemolyticus	–	–	–	–

CME: crude methanol extract; HAF: hexane fraction; EAF: ethyl acetate fraction; CHF: chloroform fraction; –: no detection of antibacterial activity.
[a] Values are represented as the mean ± S.D. of three experiments.

3.3. Minimum inhibitory concentration (MIC)

According to the results given in Table 2, MIC of essential oils and all organic extracts fraction did not show desirable results against all the bacterial tested.

4. Discussion

Since pre-historic times, man has gone in different ways to search for cures and relief from various diseases by using numerous plants, plant products and plant-derived products. Recently, there is a scientific interest and a certain popularity with regard to screening essential oils and extracts from plants used medicinally all over the world. Historically, many plants' essential oils and crude extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties (Hoffman, 1987; Lawless, 1995; Jovanovic et al., 2005; Kim and Shin, 2004; Magwa et al., 2006; Marino et al., 2001; Mitscher et al., 1987; Mourey and Canillac, 2002). It is very important now-a-days to investigate scientifically those plants, which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher et al., 1987). Also, the resurgence of interest in natural control of phytopathogens and increasing consumer demand for effective, safe, and natural products means that quantitative data on plant oils and extracts are required. Various publications have documented the anti-fungal and antibacterial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Morris et al., 1979; Yousef and Tawil, 1980).

The hydro distillation of the leaves and stems of *M. borneensis* gave dark yellowish oils with the major components of the oil having oxygenated mono and sesquiterpenes, and their respective hydrocarbons. Recently several researchers have reported that mono and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils of plant origin, which have enormous potential to strongly inhibit microbial pathogens (Gudzic et al., 2002; Cakir et al., 2004; Sacchetti et al., 2004; Sara, 2004; Shunying et al., 2005; Sur et al., 1991). In general, the active antimicrobial compounds of essential oils are terpenes, flavonoids which are phenolic in nature, and it would seem reasonable that their antimicrobial or antibacterial mode of action might be related to that of other compounds.

The essential oils of *M. borneensis* did not show any antibacterial effect against all the bacterial tested. Some earlier papers on the analysis and antibacterial properties of the essential oils of some species of various genera have shown that they have a varying degree of growth inhibition effects against some *S. aureus*, *S. typhimurium* and *Fusarium* species due to their different chemical compositions (Alvarez-Castellanos et al., 2001; Singh et al., 2002; Bouchra et al., 2003; Awale et al., 2003; Azaz et al., 2002; Filipowicz et al., 2003). Bouchra et al. recently reported that the essential oils of several Moroccan Labiatae, which consist mainly carvacrol, linalyl acetate and tymol as major components, exhibited a complete disc diffusion method on nutrient agar medium on the growth of *B. cinerea* (Bouchra et al., 2003). In spite of this, most of these oils are commercially available for purchase as whole oils or as a part of pharmaceutical or cosmetic products, indicating that toxic properties do not prohibit their use. However, our ongo-

ing investigation of toxic or irritant properties is imperative, especially when considering any new products for human use, medicinal or otherwise.

Most of the plant extracts with their derived crude fractions and phytochemicals act in many ways on various types of disease complex, and may be applied to the crops in the same way as other agricultural chemicals. *M. borneensis* can also be used as a leading factor in a wide range of activities against many phytopathogens, where the pathogens have developed resistance against the specific bactericides (Elad, 1991). In this study, the essential oils and the different extracts did not show any antibacterial activity against various plant pathogenic bacteria, which could be attributed due to low concentration of essential oil and crude extracts. Therefore, it would also be very interesting to study the effects of essential oils and crude extracts of *M. borneensis* against important bacteria for developing new anti-bacterial agents to control serious fungal diseases in plants as well as crude extracts, animals and human beings. In this regard, we have started a program aimed at the determination of antibacterial activity of essential oils and methanol extract of *M. borneensis* and its derived crude fractions of hexane, ethyl acetate and chloroform, in the hope that we would findout new natural products to be used in biocontrol against the phytopathogens.

Thus, *M. borneensis* could become an alternative to synthetic bactericides for use in agro industries and also to screen and develop such novel types of selective and natural bactericides in the treatment of many microbial phytopathogens causing severe destruction to crops, vegetables and ornamental plants.

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References

Abramowize, M., 1990. Toxic psychiatry: drugs and electroconvulsive therapy. Left Drug Therapy 32, 41–48.

Adam, R.P., 2001. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, IL, USA.

Ahmad, I., Mehmood, Z., Mehmood, I., 1998. Evaluation of antipyretic and analgesic activity of *Emblica officinalis* Gaertn. Journal of Ethanopharmacology 62, 183–193.

Ahmet, C., Saban, K., Hamdullah, K., Ercan, K., 2005. Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse. Biochemical Systematic Ecology 33, 245–256.

Alvarez-Castellanos, P.P., Bishop, C.D., Pascual-Villalobos, M.J., 2001. Phytochemistry 57, 99–102.

Anonymous, 1996. The Indian Pharmacopoeia, third ed. Ministry of Health and Family Welfare, Government of India, New Delhi.

Aswal, B.S., Goel, A.K., Patnaik, G.K., 1996. Screening of novel 1,5-benzothiazepines. Indian Journal of Experimental Biology 34, 444–467.

Awale, S., Tezuka, Y., Banskota, A.H., Adnyana, I.K., Kadota, S., 2003. Highly-oxygenated isopimarane-type diterpenes from *Orthosiphon stamineus* of Indonesia and their nitric oxide inhibitory activity. Chemical Pharmacological Bulletin 51 (3), 268–275.

Azaz, D., Demirci, F., Satil, F., Kurkc, U.M., Baser, K.H.C., 2002. Antimicrobial activity of some Satureja essential oils. Zeitschrift fur Naturforschung 57C, 817–821.

Bauer, A.W., Kirby, M.D.K., Sherris, J.C., Turk, M., 1966. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. American Journal of Clinical Pathology 45, 493–496.

Benson, M.J., 1990. Microbilogical Application, fifth ed. Brown Publ., USA, p. 134.

Bouchra, C., Achouri, M., Hassani, L.M.I., Hmamouchi, M., 2003. Chemical composition and anti-fungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. Journal of Ethnopharmacology 89, 165–169.

Cakir, A., Kordali, S., Zengin, H., Izumi, S., Hirata, T., 2004. Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. Flavour Fragmentation Journal 19 (1), 62–68.

Elad, Y., 1991. Multiple resistance to benzimidazoles dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. Plant Pathology 41, 41–46.

Filipowicz, N., Kamiński, M., Kurlenda, J., Asztemborska, M., 2003. Antibacterial and antifungal activity of Juniper Berry oil and its selected components. Phytochemistry Research 17, 227–231.

Fong, H.H.S., 1973. Phytochemical Screening. University Duplicating Series, California, p. 43.

Fransworth, N.R., Loub, W.D., 1983. In: Workshop Proceedings. OTA-BP-F-23 US Congress, Washington, DC, pp. 178–195.

Gudzic, B., Djokovic, D., Vajs, V., Palic, R., Stojanovic, G., 2002. Composition and antimicrobial activity of the essential oil of *Hypericum maculatum* Crantz. Flavour Fragmentation Journal 17 (5), 392–394.

Guillen, M.D., Manzanos, M.J., 1998. Composition of the extract in dichloromethane of the aerial parts of a Spanish wild growing plant *Thymus vulgaris* L. Flavour Fragmentation Journal 13, 259–262.

Haznedaroglu, M.Z., Karabay, N.U., Zeybek, U., 2001. Antibacterial activity of *Salvia tomentosa* essential oil. Fitoterapia 72 (7), 829–831.

Hoffman, D.L., 1987. The Herb User's Guide. Thorsons Publishing Group, Wellingborough, UK.

Hossain, M.A., Salehuddin, S.M., Ismail, Z., 2006. Rosmarinic acid and methyl rosmarinate from *Orthosiphon stamineus*, Benth. Journal of Bangladesh Academic Sciences 30 (2), 167–171.

Hunter, P.A., Reeves, D.S., 2002. The current status of surveillance of resistance to antimicrobial agents: report on a meeting. Journal of Antimicrobial and Chemotherapy 49, 17–23.

Janovska, D., Kubikova, K., Kokoska, L., 2003. In vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Czech Journal of Food Science 21, 107–111.

Jovanovic, T., Kitic, D., Palic, R., Stojanovic, G., Ristic, M., 2005. Chemical composition and antimicrobial activity of the essential oil of *Acinos arvensis* (Lam.) Dandy from Serbia. Flavour Fragmentation Journal 20, 288–290.

Karmen, V., Bojana, B., Margareta, V., Franc, P., 2003. Effect of the antifungal activity of oxygenated aromatic essential oil compounds on the white-rot *Trametes versicolor* and the brown-rot *Coniophora puteana*. International Biodetermination Biodegradation 51 (1), 51–59.

Kim, Y-S., Shin, D-H., 2004. Volatile constituents from the leaves of *Callicarpa japonica* Thunb. and their antibacterial activities. Journal of Agricultural Food Chemistry (4), 781–787.

Laven, M., Vanen Berghe, D.A., Mertens, F., 1979. Structure and chemotherapeutical activity of a polyisoprenylated benzophenone

from the stem bark of *Garcinia huillensis*. Planta Medica 36, 311–321.

Lawless, J., 1995. The Illustrated Encyclopedia of Essential Oils. Element Books Ltd., Shaftesbury, UK.

Lingk, W., 1991. Health risk evaluation of pesticide contamination in drinking water. Gesunde Pflangen 43, 21–25.

Magwa, M.L., Gundidza, M., Gweru, N., Humphrey, G., 2006. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. Journal of Ethnopharmacology 103, 85–89.

Marino, M., Bersani, C., Comi, G., 2001. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceace* and *Compositae*. International Journal of Food Microbiology 67, 187–195.

Mitscher, L.A., Drake, S., Gollapudi, S.R., Okwute, S.K., 1987. The society for applied microbiology. Journal of Applied Microbiology 86, 985–990.

Mitscher, L.A., Drake, S., Gollapudi, S.R., Okwute, S.K., 1987. A modern look at folkloric use of anti-infective agents. Journal of Natural Product 50, 1025–1040.

Morris, J.A., Khettry, A., Seitz, E.W., 1979. Antimicrobial activity of aroma chemicals and essential oils. Journal of American Oil Chemistry Society 56, 595–603.

Mourey, A., Canillac, N., 2002. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Control 13 (4–5), 289–292.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., 1995. Manual of Clinical Microbiology, sixth ed. ASM, Washington, DC.

Nelson, R., 2007. Quantification of vincristine and its major metabolite in human plasma by high-performance liquid chromatography/tandem mass spectrometry. Oncology 10, 115–127.

Olah, N.K., Radu, L., Mogosan, C., Hanganu, D., Gocan, S., 2003. Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth. (Lamiaceae) hydroalcoholic extract. Journal of Pharmaceutical Biochemistry Analysis 33, 117–123.

Ormancey, X., Sisalli, S., Coutiere, P., 2001. Formulation of essential oils in functional perfumery. Parfums, Cosmetiques, Actualites 157, 30–40.

Oumzil, H., Ghoulami, S., Rhajaoui, M., Ilidrissi, A., Fkih-Tetouani, S., Faid, M., Benjouad, A., 2002. Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*. Phytochemical Research 16, 727–731.

Pattnaik, S.S., Bapaji, J., Kole, C.R., 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbiology 358, 39–46.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 269, 337–341.

Radja Commare, R., Nandakumar, R., Kandan, A., Suresh, S., Bharathi, M., Raguchander, T., Samiyappan, R., 2002. *Pseudomonas fluorescens* based bio-formulation for the management of sheath blight disease and leaffolder insect in rice. Crops Protection 21, 671–677.

Sacchetti, G., Medici, A., Maietti, S., Radice, M., Muzzoli, M., Manfredini, S., Braccioli, E., Bruni, R., 2004. Composition and functional properties of the essential oil of Amazonian Basil, *Ocimum micranthum* Willd., Labiatae in comparison with commercial essential oils. Journal of Agriculture Food Chemistry 52 (11), 3486–3491.

Samy, R.P., Ignacimuthu, G., Sen, A., 1998. In-vitro cytotoxicity (LC50) of extracts obtained from the seeds of *Zea mays*. Journal of Ethnopharmacology 62, 172–182.

Sara, Burt, 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology 94 (3), 223–253.

Sawamura, M., 2000. Aroma and functional properties of Japaneseyuzu (*Citrus junos* Tanaka) essential oil. Aromatic Research 1 (1), 14–19.

Schneider, G., Tan, H.S., 1973. Isolation and characterisation of flavonoids. Deatsche Apotheker Zeitung 113, 201.

Schumutterer, H., 1990. Further studies on mating disruption in pink bollworm, *Pectinophora gossypiella* (Saunders) using sex pheromone as a component of IPM programme in irrigated cotton fields in Punjab *Azadirachta indica* Ann. Review Entomology 35, 271–273.

Schut, G.A., Zwaving, J.H., 1993. Pharmacological investigation of some lipophilic flavonoids from *Orthosiphon aristatus*. Fitoterapia 64, 99–102.

Shunying, Z., Yang, Y., Huaidong, Y., Yue, Y., Guolin, Z., 2005. Chemical composition and antibacterial activity of the essential oils of *Chrysanthemum indicum*. Journal of Ethnopharmacology 96, 151–158.

Singh, G., Singh, O.P., Maurya, S., 2002. Chemical and biocidal investigations on essential oils of some Indian *Curcuma* species. Program Crystal Growth and Characterization 45, 75–81.

Stecher, G.P., 1976. The Merck Index, 9th ed. p. 9552.

Sur, S.V., Tuljupa, F.M., Sur, L.I., 1991. Gas chromatographic determination of monoterpenes in essential oil medicinal plants. Journal of Chromatography 542, 451–458.

Tezuka, Y., Stampoulis, P., Banskota, A.H., Awale, S., Tran, K.Q., Saiki, I., 2000. Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*. Chemical Pharmaceutical Bulletin 48, 1711–1719.

Yousef, R.T., Tawil, G.G., 1980. Antimicrobial activity of volatile oils. Die Pharmaceutical 35, 698–701.