The Government of the Union of Myanmar
Ministry of Education

Department of Higher Education (Lower Myanmar)
and
Department of Higher Education (Upper Myanmar)

Universities Research Journal

Vol. 1, No. 3 December, 2008.

Universities Research Journal 2008
Vol. 1, No. 3

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Contents

	Page
Determination of Calorie Contents of Myanmar Snacks from Thanlyin Township *Nyo Nyo Aung, Mya Mya Mu and Myat Sandar Hla*	1
Analysis of the Carbonate in Limestone from Loikaw Area *Tha Zin, Than Than Myint and Ni Ni Sein*	19
Preparation and Application of Intercalated Zinc Oxide Carbon Molecular Sieves *Mya Thuzar, Nyunt Wynn and Khin Mar Tun*	31
Process Development of Lentil flour- based Adhesive for Woodworking Industries *Tin Sein*	39
Isolation, Identification and Antibacterial Activity of Some Xanthones Present in Fruit Hulls of *Garcinia Mangostana* Linn. *Sandar Aung, Aye Aye Tun, San San Aye, and Maung Maung Htay*	47
Studies on an Unknown Compound from *Argyreia barbigera* Choisy *Htay Htay Win*	57
An Antioxidant Organic Compound Isolated from the Stem of *Hypericum calycinum* L. (Pyin-nyar-lin-kar) *Thida Win, Thant Thant Htwe, Myint Myint Sein and Joerg Heilmann*	71
Repellent Action of Citronella Oil Against *Aedes Aegypti* Mosquito *Ei Ei Soe*	81
Investigation of the Antioxidant Activity of *Cydonia cathayensis* Hemsl. (Chinsaw-ga) Fruit *San San Oo*	95
Isolation and Structural Elucidation of an Unknown Biologically Active Compound from Myanmar Traditional Indigenous Medicinal Plant *Clerodendrum serratum* SPRENG (Yin-bya-net) *Aye Myint, Myint Myint Sein and Mya Aye*	105

Page

Anti-*Helicobacter pylori* and Anti-tumor Activities of (Korea and Myanmar) Herbal Medicines 119
Hnin Hnin Aye

Structural Elucidation of a Bioactive Carbazole Compound Isolated from *Pteris pellucida* Presl. (Say-ba-zun-doke) 129
Lwin Lwin Myint

Thermodynamic Investigation of Dodecylpyridinium Ion Binding with Fulvic and Humic Acids 141
Min Min Yee, Tohru Miyajima and Noboru Takisawa

Detecting the Incoming Objects by Using Infrared Radiation 151
Moe Nyo, Than Tun Oo and Aye Maw Aung

High Performance Computing in Yangon University 161
Pho Kaung and Ye Chan

Peripheral Interface Controller - Based Frequency Meter 167
Htar Htar Aye Win, Thida Soe and Ni Ni Yin

Spectral Analysis on Voices of Myanmar Characters and Words 177
Ye Chan and Win Win Kyi

Polarization Phenomena Associate with Scattering of Radiation in Astrophysics 187
Yee Yee Oo, G. Padmanabha and G. Ramachandran

The Study of the Curves of Functions for Data Points 203
Hla Myint Kyaw

Analysis of a Double-□ Hypernucleus Event in the KEK-PS E373 Experiment 217
Khin Than Tint

Elemental Analysis of Tawkyetthun (Herbal Plant) for Treatment of Diabetes 227
Khin Tint

Production of Kaonic Nuclei K^-pp by $p(p, K^+)$ and $p(d, K^0)$ Reactions 235
Htar Win Htike, Mar Mar Htay and Khin Swe Myint

	Page
Study on the Number of Alpha Tracks and Pore Diameters Based on Annealing Method *Mya Mya Win*	247
Charge Storage Mechanism of $PbTi_{0.99}Al_{0.01}O_3$ Gated Short – Channel Thin Film Transistor *Khin Nyo Win, Kyi Kyi Aung and Lai Lai Aung*	259
Effects of Overgrowth, Growth Rate, and Capping of InAs Quantum Dots Grown on Cross-hatch Surfaces by Molecular Beam Epitaxy *Cho Cho Thet, Ko Ko Kyaw Soe, Teeravat Limwongse, Somsak Panyakeow and Songphol Kanjanachuchai*	269
Electrical Properties of $Zn_{1-x}Cu_xO$/Si Thin Film *Min Maung Maung and Aye Myat Minn*	277
Growth and Characterization of Indium doped Zinc Oxide Solar Cell *Yee Yee Oo, Aye Aye Swe and Than Than Win*	285
Growth Mechanism, XRD, Raman and FTIR Spectroscopic Studies of Potassium Pentaborate (KB5) Crystal *Zin Min Tun and Win Kyaw*	293
Growth of Sol-Gel Derived Lead Titanate Thin Film for Non-Volatile Memory Device Application *Khin Moe Thant and Yin Maung Maung*	303
Ionic Conductivity and Dehydration of $ZnSO_4.7H_2O$ Crystal at High Temperature *Wut Hmon Win*	313
Ionic Conductivity of xM_2O . $(1-x)$ B_2O_3 Glass *Soe Soe Thin*	325
Study on Ferroelectric Properties of TiO_2 / SiO_2 /p-Si (Metal/ Ferroelectric/ Insulator/Semiconductor) Thin Films *May Yee Thein, Yin Yin Thein, Than Than Win and Ko Ko Kyaw Soe*	335
Multi-Agent Architecture Approach to Web-Based Teaching System *Nwe Nwe Win*	345
Optimizing Database Queries by Indexing *Soe Mya Mya Aye*	355

Isolation, Identification and Antibacterial Activity of Some Xanthones Present in Fruit Hulls of *Garcinia Mangostana* Linn.

Sandar Aung[1], Aye Aye Tun[2], San San Aye[3], and Maung Maung Htay[4]

Abstract

The fruit hulls of mangosteen, *Garcinia mangostana* Linn. (Guttiferae), have been in use in Myanmar folk medicine for the treatment of skin infections, wounds, diarrhea and dysentery. The main aim of this work was the use of mangosteen in traditional medicine to get scientific scrutiny. Phytochemical investigations on mangosteen fruit hulls indicated the presence of carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, flavonoids and tannins in it. Four xanthones, namely, gartanin (1) (m.p 146-152°C; yield 0.48%), and compound mangostin (2) (m.p 179-181°C, 0.97% yield), β-mangostin (3) (m.p 175°C, 0.063% yield) and nor-mangostin (4) (m.p 196-198°C, 0.91% yield) were isolated and spectroscopically identified from the fruit hulls of mangosteen collected from Kyeik-kaw township of Mon State in June, 2003. Antibacterial activities of crude extracts (petroleum ether and ethanol extract) by agar disc diffusion method against 33 bacteria strains including *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Staphylococcus aureus, Bacillus substilis, Klaebsiella aerginosa, Proteus morganii, Plesiomonas shigelloides, Pseudomonas pyocyanea* and *Vibrio* spp. were evaluated. Ethanol extract inhibited the growth of *B. substilis* and *S. aureus* as evidenced by zone inhibition diameter measurements (d=14mm for both strains) when compared to standard antibiotic. In addition, 1 and 2 exhibited inhibitory effect against resistant strains of *Staphylococcus aureus* with the minimum inhibitory concentration (MIC) value of >0.1 mg/ml and 0.05 mg/ml, respectively, showing the superiority of 2 over 1. No inhibitory effect was observed against remaining strains indicating narrow antibacterial spectrum of mangosteen crude extract and its constituents. *S. aureus* and its pathogenic capacity were mentioned that the effect of food poisoning, boils, abscesses and wound sepsis. This study proved mangosteen fruit hulls which are widely used in remedies of traditional medicine are of valuable antibacterial agents especially for *S. aureus* infection. It may be used in the treatment of amoebic dysentery; however, it should not be used for bacillary dysentery which is caused by members of the genus *Shigella*.

Key words: mangosteen, *Garcinia mangostana* Linn, *S. aureus* infection, **amoebic** dysentery, gartanin, mangostin, β-mangostin, nor-mangostin.

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Introduction

Today, natural colourants are emerging globally, leaving synthetic colourants behind in the race, due to the realization that are safer and ecofriendly in nature. The use of synthetic colourants is gradually receding due to an increased environmental awareness and harmful effects because of either toxic degraded products or their non-biodegradable nature. Recently, a ban has been imposed all over the world on the use of some synthetic colourants which finally triggered active research and development to revive world heritage and traditional wisdom of employing safer natural colourants. A few examples of plant natural colouring matters are logwood, annatto, madder, indigo, safflower, quercitron, and tumeric (Kirk, 1950). The deep red colouring matter, the dye obtained from annatto is used in traditional medicine as a purgative, astringent, antileprotic, antiemetic and to treat blood and kidney diseases (Kanjilal, 1995). The colouring matters of animal origin are cochineal, kermes and lac dyes (Triebold, 1963). The principal pigment in cochineal is a protein bound glycoside of the anthraquinone, carminic acid. Treatment of carminic acid with and aluminium salt produces a soluble aluminium lake, known as carmine. Carmine (red colouring matter) is the main product employed for cosmetics, food and pharmaceutical colouring applications (website 1). Blue from indigo, yellow from tumeric and saffron, brown from cutch and red from lac, safflower and madder are commonly known. Thus, natural colourants have been an integral part of human life (Kumar, 2004).

Generally natural colourants do not cause health hazards and act as a health cure like rathanjot, tumeric etc. (Kumar, 2004). Ranthanjot, a red dye from roots, is not only used for food staffs but also as medicine to treat eye diseases, bronchitis, abdominal pains, anthelmintic, antipyretic and antiseptic properties (Khatoon, 1993). Yellow dye from rhizomes of tumeric is traditionally used in medicine as an anti-inflammatory drug (Shah, 1997). Similarly, Mangostin, a yellow colouring matter, obtained from the fruit hulls of *Garcinia mangostana* Linn. (mangosteen) is used as astringent and also used against cholera, dysentery and diarrhoea (Sen *et al*, 1980). The fruit hull of mangosteen has been used many years as a medicine for treatment of skin infection, wounds and diarrhoea in Southeast Asia (Nakatani, 2002).

Myanmar people also traditionally employ a decoction of the hulls and bark as a febrifuge and to treat diarrhoea, dysentery and urinary

disorders (Yates, 1958). The chemical constituents present in mangosteen may depend on its origin and maturation of the fruit. Because of growing interest in correlating phytochemical constituents of a plant with its pharmacological activity (Gupta, 1994), it is essential to investigate the bioactivity and chemical constituents present in the fruit hulls of mangosteen.

Materials and Methods

General procedures.

Mps: uncorr; 1H (400 MHz) and 13C (100 MHz) NMR: BRUKER DPX -400, $CDCI_3$ with TMS as int. standard; UV: shimadzu UV-240, cyclohexane; FT-IR: Perkin Elmer GX system, KBr; GC-MS: Perkin Elmer GCMS; CC: Merck silica gel 60 (70-230 mesh), eluents: petroleum ether-ethyl acetate (PE-EtOAc),TLC: 0.25 mm precoated silica gel (60 F254, Merck), solvent system: 1 toluene:ethyl acetate (4:1) and 2.Ethyl acetate: pet ether (3:1), 3. Chloroform: methanol (19:1),4. Chloroform: methanol (19:1),spots were detected by inspection under UV light (254mm or 365 nm) or by the colour developed with 5% sulpuric acid spraying followed by heating.

Plant Materials.

Mangosteen used in this study were collected from Kyeik-kaw in Mon State in June, 2003.

Preliminary Phytochemical Examination of Sample

Preliminary phytochemical examinations were done on this sample according to the standard methods as follows: Test for carbohydrate, Test for glycosides, Test for organic acid, Test for phenolic compounds, Test for α-amino acid, Test for alkaloids, Test for steroids, Test for flavonids and Test for cyanogenic glycoside.

Extraction and Isolation of Compounds

Procedure

The air-dried powdered fruit hulls (100g) were extracted with pet ether (60-80.) (600ml)for 8 hours in a soxhelt extractor. Removal of the solvent provided crude extract (1.00g), which was chromatographed on silica gel (50g, d=2cm, 1=20cm) in pet ether; The coloumn was eluted with increasing amount of ethyl acetate in pet ether; PE - EtOAc (9:1), PE-

EtOAc (17:3), PE-EtOAc (4:1). 10cm3 in each fraction were collected and the chromatography was monitored by TLC using 1 : PE-EtOAc (1:3)V/V; 2: PE-EtOAc (3:1) V/V solvent system. The fractions that gave similar TLC pattern were combined together and concentrated. Compound 1 (gartanin): yellow needles and the yield was 0.48% (23.1 mg). Compound 2 (mangostin): orange needles and the yield was 0.97% (46 mg) based upon the crude extract.

The air-dried powdered fruit hulls (100.0g) were extracted with ethanol (600 ml)for 8 hours in a soxhlet extractor. The ethanol extract was exhautively dissolved in ethyl acetate and then filtered Evaporation of ethyl acetate provided the crude extract, which was chromatographed in silica gel (50g, d=2cm, l=20cm) in PE-EtOAc (7:1). The column was filled with the solvent system and fractions were collected at the rate of one drop per second. The fractions that gave similar TLC pattern were combined together and concentrated and compound 3 (b - mangostin): lemon yellow solid and the yield was 0.063% (5mg) and compound 4 (Nor-mangostin): yellow solid and the yield was 0.91 % (73 mg) based upon the crude extract.

Screening of Antibacterial Activities of 70 Percent Ethanol Extracts by Agar Disc Diffusion Method

Screening of antibacterial activities of ethanol extract was done by agar disc diffusion method. In the present work, the samples were tested for antibacterial activity against 33 strains. The tested bacteria included 7 species of each Escherichia coil and Shigella; 6 species of Salmonella; 4 species of each Staphylococcus aureus and Vibrio; and 1 species of each Bacillus subtilis, Klebsiella aerginosa, Proteus morganii, Plesiomonas shigelloides and Pseudomonas Pyocyanea.

Determination of Minimun Inhibitory Concentration (MIC) of Isolated Xanthones by Microplate Dilution Method

Bacteriostatic and bactericidal activities of the isolated compounds against S. aureus (1), (2), (3), (4) were tested by microplate dilution method.

Results and Discussion

Preliminary Phytochemical investigation:

Carbohydrate, glycoside,1 flavonoid, organic acid, phenolic compound, reducing sugar and tannin were found to be persent.a-amino acid, alkaloid, steroid, and cyanogenic glycoside were found to be absent.

Identification of Compounds

The pet ether extract on chromatographic separation and further purification produced two xanthones, gartanin (1) and mangostin (2) Mangostin was isolated a major constituent from PE extract Garcinia mangostana. Chromatographic separation of ethyl acetate soluble portion of Ethanol extract and further purification provided two xanthones β-mangostin (3) and nor-mangostin (4). The chemical and spectral data of four xanthones are shown as follows.

Gartanin (1). C23 H24 O6, yellow needles (23.1 mg, 0.48% yield); m.p 1460-1520C (Lit. 1670C, Govindachari et al, 1971); λmax 240, 260, 281, 350 nm (lit. 259, 284, 325 (sh), 35lnm. Govindachari et al, 1971); λmax 3419 (nO-H), 2969 (nC-H), 1628, (nC=0), 1376 (dCH3) and 1284 (nar C-O); d(CDCl3+DMSO-d6) 12.3 (1H,s), 11.3 (1H,s), 7.21(1H, d, J - 9Hz), 6.25 (1H , d, J = 9Hz), 5.4-5.2 (2H, m), 3.52-2.95(4H, br), 1.80 (12 H, s) and 1.70 (12 H, s); dC 116.874 (-CH = CH2) 121.087 (aromatic C-H), 130.046 (aromatic C-C), 135.797 (-CH = CH2), 142.946 (aromatic C-O-CH3); EI - MS m/z (%) 396 (M+) 381, 353, 341, 325, 297, 285 (100%) (Lit. 396 (M+), 381, 353, 341, 325, 297, 285 (100%), 381, 379, 353, 341, 325, 297, 285, Govindachari et al, 1971.

Mangostin (2). C23 H24 O6, orange needles (46 mg, 0.97% yield); m.p 1790-1810C (Lit. 1810C, Govindachari et al, 1971); λmax 245, 261, 308, 353 nm (lit. 243, 259, 318 , 351 nm. P.Yates and H.stout et al, 1958); λmax 2928 (C-H), 1622 (C=C), 1185, (C -OH), 841cm-1 (C-H) and; dH 13.7 (1H,s), 6.7 (1H,s), 6.35(1H, s), 5.25 (2H, m), 4.2 (2H, d, J=9Hz), 3.25(2H, d, J= 9Hz),1.69-1.64(3s, 12 H) ; dC 62.065 (R-O-CH3), 112.596(aromatic C-H), 121.490(aromatic C-H), 132.624 (aromatic C), 137.097 (-CH=CH2); 142.699 (aromatic C-O-CH3); EI - MS m/z (%) 410 (M+) 382, 367, 354, 339 (100%), 311, (Lit. 410 (M+), 393, 367, 355, 354, 339 (100%), (Alfred Wan, 1973).

β Mangostin (3): C25 H28 O6 Lemon yelow solid (5mg, 0.063% yield) m.p 1750 C (lit. 175oC, Jefferson, 1970), λmax (cyclohexane) 209, 243, 259 and 315nm (lit. 212, 244, 259, 315, 349 nm, Jefferson, 1970); n max (KBr) 3457 (nO-H), 2922 (nc - H) 1460 (c = c), 1280 cm-1 (nar C-O).

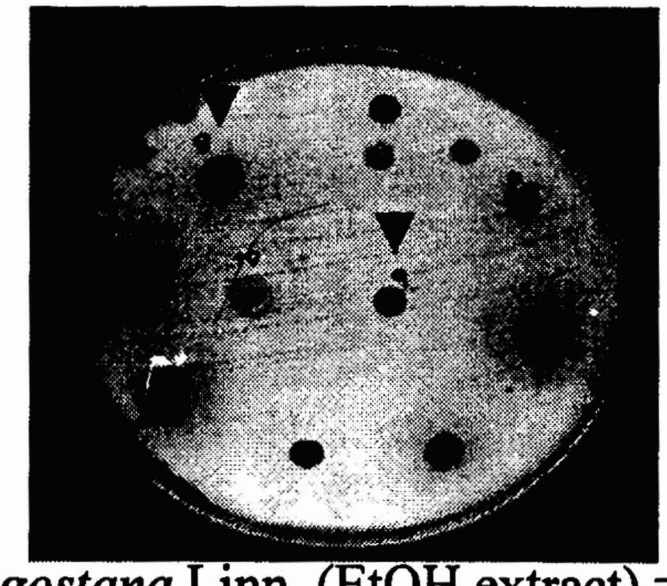
Nor-mangostin (4) : C23 H24 O6, yellow solid (73 mg, 0.91% yield); m.p 1960-1980C (Lit. 1980C, Govindachari et al, 1971); lmax 236, 260, 219, and 378 nm (lit. 243, 260, 317 and 360 nm, Govindachari et al, 1971); lmax 3407 (nO-H), 2921 (nC-H), 1642, (nC=0), 1619 (C=C) and 1281cm-1 (nar C-O); dH(CDCl3) 13.7 (1H,s), 6.79 (1H,s), 5.30(2H, br), 4.22 (2 H, br) and 3.45 (2 H, br); 1.88 (3H, s) and 1.77 (3H, s).

Screening of Antibacterial Activities of 70 Percent Ethanol Extracts by Agar Disc Diffusion Method

Ethanol extract inhibited the growth of *B.substilis* and *S. aureus* as evidenced by zone inhibition diameter measurements (d= 14mm for both strains) when compared to standard antibiotic.

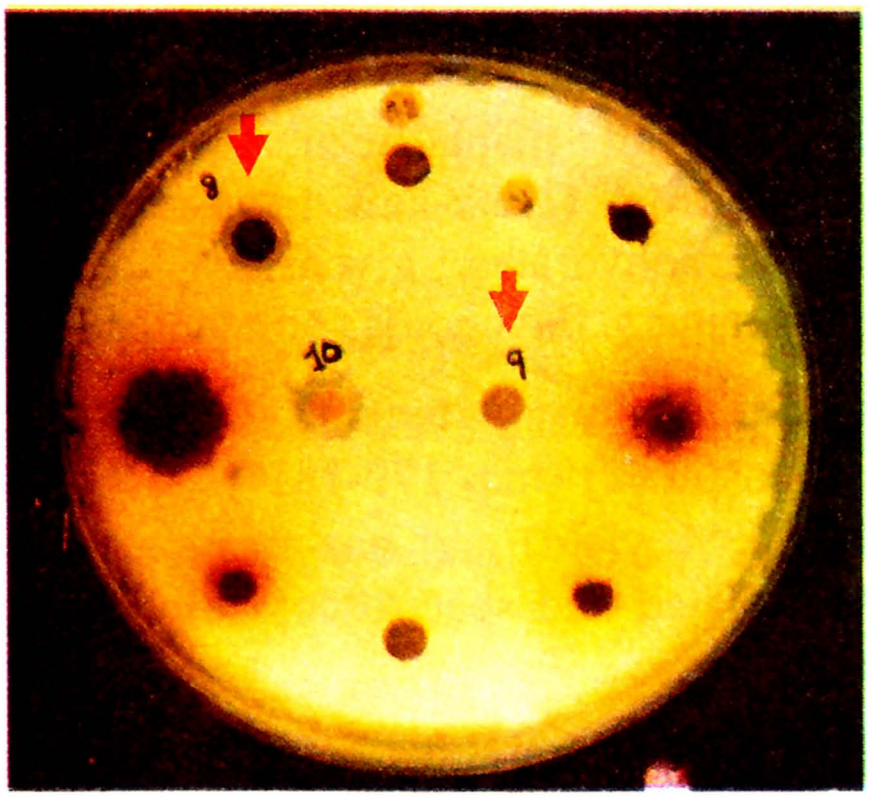
Determination of Minimum Inhibitory Concentration (MIC) of Isolated Xanthones by Microplate Dilution Method

The minimum inhibitory concentration (MIC) value of gartanin (1) was found to be > 0.1 mg/ml while mangostin (2) had MIC = 0.05 mg/ml. This means that mangostin (2) is more active than gartanin (1) against tested organisms.



8. *Garcinia mangostana* Linn. (EtOH extract) = 14 mm
9. *Garcinia mangostana* Linn. (PE extract) = (-) ve Disc diameter = 6 mm

Figure 1. Effect of crude extracts of *Garcinia mangostana* Linn. (mangosteen) fruit hulls on *Staphylococcus auraus.*



8. *Garcinia mangostana* Linn. (EtOH) = 14 mm
9. *Garcinia mangostana* Linn. (PE) = (-) ve Disc diameter = 6 mm

Figure 2. Effect of crude extracts of *Garcinia mangostana* Linn. (mangosteen) fruit hulls of on *Bacillus subtilis*.

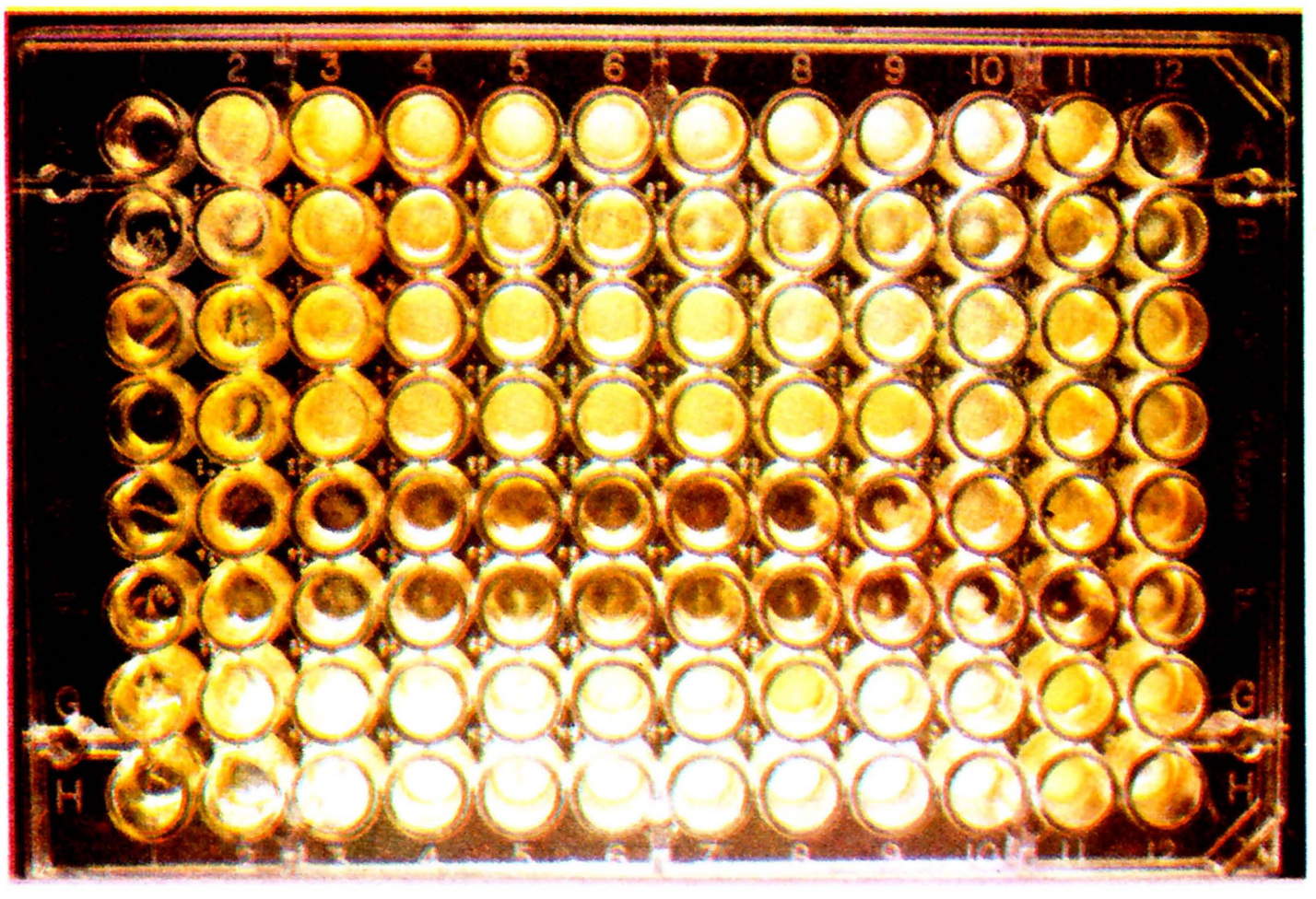


Figure 3. Minimum inhibitory concentration (MIC) of gartanin 1 and mangostin 2 of fruit hulls of *Garcinia mangostana* Linn. against *Staphylococcus aureus* (1), (2), (3), (4) by microplate dilution method

Conclusion

The fruit hulls of mangosteen, *Garcinia mangostana* Linn. (Guttiferae), collected from Kyeik-kaw township of Mon state in June, 2003, consists of carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, flavonoids and tannins.

Four xanthones, namely, gartanin (1) (m.p 146-152°C; yield 0.48%), and compound mangostin (2) (m.p 179-181°C, 0.97% yield), β-mangostin (3) (m.p 175°C, 0.063% yield) and nor-mangostin (4) (m.p 196-198°C, 0.91% yield) were isolated and spectroscopically identified from the fruit hulls of mangosteen.

In vitro antibacterial activities of crude extracts (petroleum ether and ethanol extract) against 33 bacteria strains including *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Staphylococcus aureus, Bacillus substilis, Klaebsiella aerginosa, Proteus morganii, Plesiomonas shigelloides, Pseudomonas pyocyanea* and *Vibrio* spp. were evaluated by agar disc diffusion method. Ethanol extract inhibited the growth of *B. substilis* and *S. aureus* as evidenced by zone inhibition diameter measurements (d=14mm for both strains) when compared to standard antibiotic.

1 and 2 exhibited inhibitory effect against resistant strains of *Staphylococcus aureus* with the minimum inhibitory concentration (MIC) value of >0.1 mg/ml and 0.05 mg/ml, respectively, showing the superiority of 2 over 1.

No inhibitory effect was observed against remaining strains indicating narrow antibacterial spectrum of mangosteen crude extract and its constituents. This study proved mangosteen fruit hulls which are widely used in remedies of traditional medicine are of valuable antibacterial agents especially for *S. aureus* infection such as food poisoning, boils, abscesses and wound sepsis. Indeed, 2 was the most effective antibacterial agents regarding to *in vitro* antibacterial activity test.

The mangosteen fruit hulls and its constituents (1 and 2) may be used in the treatment of **amoebic dysentery as claimed by the traditional medicinal practitioners;** however, it should not be used for bacillary dysentery which is caused by members of the genus *Shigella*.

Acknowledgements

The author gratefully thanks to Professor U Kyaw Myint Oo, Rector of Dagon University for giving opportunity to present this paper. I would like to thank Dr. Aye Aye Tun, Associate Professor, Department of Chemistry, Yangon University for her advice and suggestion.

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