**Medicinal use compared with Laboratory research: Relation of traditional sceince and modern science**

**by Brendan Walshe Rouusel**

## Introduction

Official recognition of traditional medicine by the local medical community is hampered by lack of validation of safety and efficacy of traditional medicine. In our retrospective study with the healers, one of their most frequent requests was the scientific validation of their traditional medicine, so that it receives the respect of the western trained medical community. We therefore proposed the use of quantitative ethnobotany to select key medicinal plants with the healers for validation of medicinal activity at the University of Ottawa Medicinal Plant laboratory. In the present study we evaluated ethnobotanical selected products for inflammatory conditions, since the Kekchi healers have considerable expertise in this area and it is also considered an appropriate area for self medication by regulatory authorities. The second was a broad search of other agents including topical anti-infective agents, such as products used for minor fungal or bacterial infections like athletes foot, or impetigo which are also widely and successfully used by the healers. We tested the hypothesis that healer consensus is a good predictor of medicinal activity

## Methods

*Plant Extraction techniques*

To obtain a compete spectrum of relevant phytochemicals, both water and 95% ethanol extracts were prepared. Most indigenous botanical therapies are prepared by water extraction, often involving a lengthy simmering or boiling period. Ethanolic extracts are a staple of modern extraction techniques, as the extract produced contains a wide array of phytochemicals of medium

polarity. In order to ensure extract standardization, raw plant material was extracted at a constant ratio of 1g of plant material to 10 ml of extraction solvent. The boiled water extract and the 95% EtOH extract were extracted in duplicate, and underwent sonnication, rotoevaporation, and lyphophillization. Standardized extracts were made by accurately redissolving freeze-dried extracts in water and ethanol.

*Cell culture*

THP-1 cells (www.ATCC.org, cell culture TIB-202), an immortal human monocyte line, were cultured in RPMI 1640 media (Invitrogen, Mississauga, Ontario) with 1% 0.05mM beta-mercaptoethanol, 1% penstrep (Invitrogen, Mississauga, Ontario), 10% fetal bovine serum (Invitrogen, Mississauga, Ontario) and 1% 1.0mM MEM sodium pyruvate, in a 37°C humidified environment with 5% $CO_2$.

*Cytotoxicity*

Extract toxicity will be established using Promega's CytoTox 96® Non-Radioactive Cytotoxicity Assay (Madison, Wisconsin), which examines the release of lactate dehydrogenase as an indicator of cell viability.

*Pro-inflammatory and anti-inflammatory assay*

$3 \times 10^5$ THP-1 cells are added to the wells of a 96-well plate, followed by the addition of botanical water and ethanol extracts of various concentrations for a total volume of 300ul/well. In both pro- and anti-inflammatory bioassays a botanical and a non-botanical positive control were used. With the ethanolic treatments, final EtOH concentration in the wells is 0.5%. In the pro-inflammatory assays, cells are then incubated for 24 hours. In the anti-inflammatory assays, cells are incubated for 1 hour, then stimulated with 1ug/ml LPS and allowed to incubate for 24 hours. After incubation, all cells are centrifuged at 12 000g for 10 minutes at 20°C. Cell culture pellets and supernatants are separated and stored at -80°C for subsequent analysis. ELISA kits (R & D Systems, Minneapolis, Minnesota) were used according to the manufacturer's protocol to analyze TNF-α levels in pro- and anti-inflammatory macrophage bioassay supernatants.

*Microbial strains and inoculum preparation*

All yeast like fungi are all opportunistic humain pathogens (Table 3). Fungi were cultured on Sabouraud dextrose agar medium at 30 ºC. Prior to the assay, yeast-like fungi were grown in a liquid Sabouraud dextrose broth at 30 ºC. The culture was subsequently adjusted to an $O.D._{600}$ of 2.0 and diluted to 1:200 with Sabouraud dextrose broth.

There were three gram-positive and three gram-negative bacteria, all of whom were both pathogenic and non-pathogenic (Table 3). Bacteria were cultured on Lennox broth base agar medium at ~37 ºC. Prior to the assay, they were grown in a liquid Lennox broth base at 30 ºC and subsquently diluted to 1:9 with the same broth.

All microbial cultures were kept in M.L. Smith's laboratory at Carleton University (Ottawa, ON, Canada) and all transfers were

done under sterile conditions and carried out under a biological containment hood.

**Table 3** - Source and infection type for the microbial strains used in this study.

| Species/strain | Source[1] | Infection types |
|---|---|---|
| Fungi | | |
| *Candida albicans* (Robin) Berkhout | | Systemic and subcutaneous |
| al-1 (wild type) | OGH-308-1329 | |
| CN1A (erg-, azole resistant) | N. D. Lees | |
| D10 (erg-, azole resistant) | N. D. Lees | |
| *Cryptococcus neoformans* (Sanfelice) Vuillemin | OMH-FR2704 | Systemic and subcutaneous, menigingoencephalitis |
| *Saccharomyces cerevisia* | OCI-S288c | Systematic, in immunocompromised patients |
| *Wangiella dermatitidis* (Kano) McGinnis | OMH-FR2236 | Cutaneous, and central nervous system |
| Gram-positive Bacteria | | |
| *Bacillus subtilis* (Ehrenberg) Cohn | ATCC 23857 | |
| *Enterococcus faecalis* (Andrews and Horder) Sckleifer and Kilpper-Balz | ATCC 29055 | |
| *Listeria innocua* Seeliger | ATCC 51742 | Non-pathogenic, related to *L. monocytogenis* which causes listeriosis |
| Gram-negative Bacteria | | |
| *Escherichia coli* (Migula) Castellani and Chalmers | AB 1157 | Unirary tract; diarrheal, renal, neurological complications |
| *Pseudomonas putida* (Trevisan) Migula | ATCC 12633 | |
| *Providencia stuartii* | ATCC 33672 | |

[1]OGH, Ontario General Hospital, Ottawa ON Canada; N.D. Lees, IUPUI, Indianapolis IN USA; OMH, Ontario Ministry of Health, Toronto ON Canada; OCI, Ontario Cancer Institute, Toronto ON Canada, ATCC American type culture collection.

*Disk diffusion bioassays*

100 µL of diluted culture broth was spread onto Sabouraud dextrose agar plates and Lennox broth base agar plates, for fungi and bacteria respectively, using a sterile bent metal rod. Three sterile 3M Whatman paper disks (7.5 mm diameter) were impregnated in increments with 2 mg of crude extract dissolved in solvent and placed face down on the inoculated surface after solvent was allowed to evaporated and the disk completely dry. Plates were incubated at 30 ºC for two days and zones of inhibition measured in diameter. Two known antifungal were used as positive controles: ketoconazole at 0.5 mg and berberine at 2 mg. The first is a compound chemically synthesized and the second is a natural product drug produced by many plants of the Berberidaceae and Ranunculaceae family. The solvent, 80 % ethanol, was used as the vehicle control.

This procedure differed to bacteria in extract concentrations and positive controls used. Paper disks were impregnated with 20 µg of crude extracts. Positive controls were chloramphenicol, an antibiotic isolated from cultures of *Streptomyces venezuelae* and ampicillin, a penicillin produced by semi-synthesis, were used at the same concentrations as crude extract. Plates were then incubated at 37 ºC for one day and zones of inhibition measured in diameter.

## Results

*Pro-inflammatory and anti-inflammatory assay*

16 ethanol extracts have been assayed for immunomodulatory activity (figure 1). Six extracts displayed important anti-inflammatory activity: Chacbolie kejen, Kum pim, Roc chit cuan, Xa'ab maus, Birritaq, and Ik kejen. Of particular interest, Xa'ab

maus, Birritaq, and Ik kejen are capable of returning TNF-alpha levels to that of the unstimulated control. At the highest concentration tested, these extracts display activity similar to that of the positive control, parthenolide.

16 ethanol extracts were also assayed for pro-inflammatory activity (figure 2). Three extracts displayed moderate pro-inflammatory activity: Ixcua ajaw chan, Bak nel k'ejen, and Ixcua' li k'uch. At the highest concentration tested, these extracts were able to stimulate the production of TNF-alpha to approximately ¾ the level seen in the positive control *Echinacea purpurea*.

*Disk diffusion bioassays* (Michel Rapinski)

21 ethanol extracts have been assayed for anti-microbial activity. Results in table 4 show a great diversity of inhibition between all plant species and all fungal species. Multiple comparisons show that most of the plants with inhibitory activity find themselves within a range that is statistically insignificant from the majority of the other plants. Although their zones of inhibition do not match up to those of the positive controls, their value often exceeds a quarter of those of the pure compound controls. In certain instances, such is the case for *C. albicans* D10, *C. albicans* Al1 and *C. neoformans*, the inhibition of the crude plant extracts may sometimes exceed half of that of the positive controls. In three fungal species, one plant, Ik Kejen, showed inhibition comparable to those of the controls and in all fungal species, maintained the largest zones of inhibition.

Results in table 5 shows that crude extracts were active against one gram-positive bacterium, *E. faecalis*, and two gram-negative bacteria, *P. putida* and *P. stuartii.* Zones of inhibition nearly half of those of the positive controls. Zones of inhibition for the controls, on the other hand, were also variable. Ampicillin showed little or no inhibition in three bacteria species, including *P.*

*stuartii*, which simply indicates the bacteria's resistance. But what is more surprising is that crude extracts which showed inhibitory activity for *P. stuartii* had zones of inhibition which were statistically insignificant from chloramphenicol.

*Future work*

The remaining plant extracts are in the process of being evaluated for their immunomodulatory activity. These laboratory experiments will be carried out over the next year with the funding of NSERC.

**Figure 1 –** Anti-inflammatory activity of ethanol extracts from 16 immunomodulatory plants used by the Q'eqchi Maya of Belize.

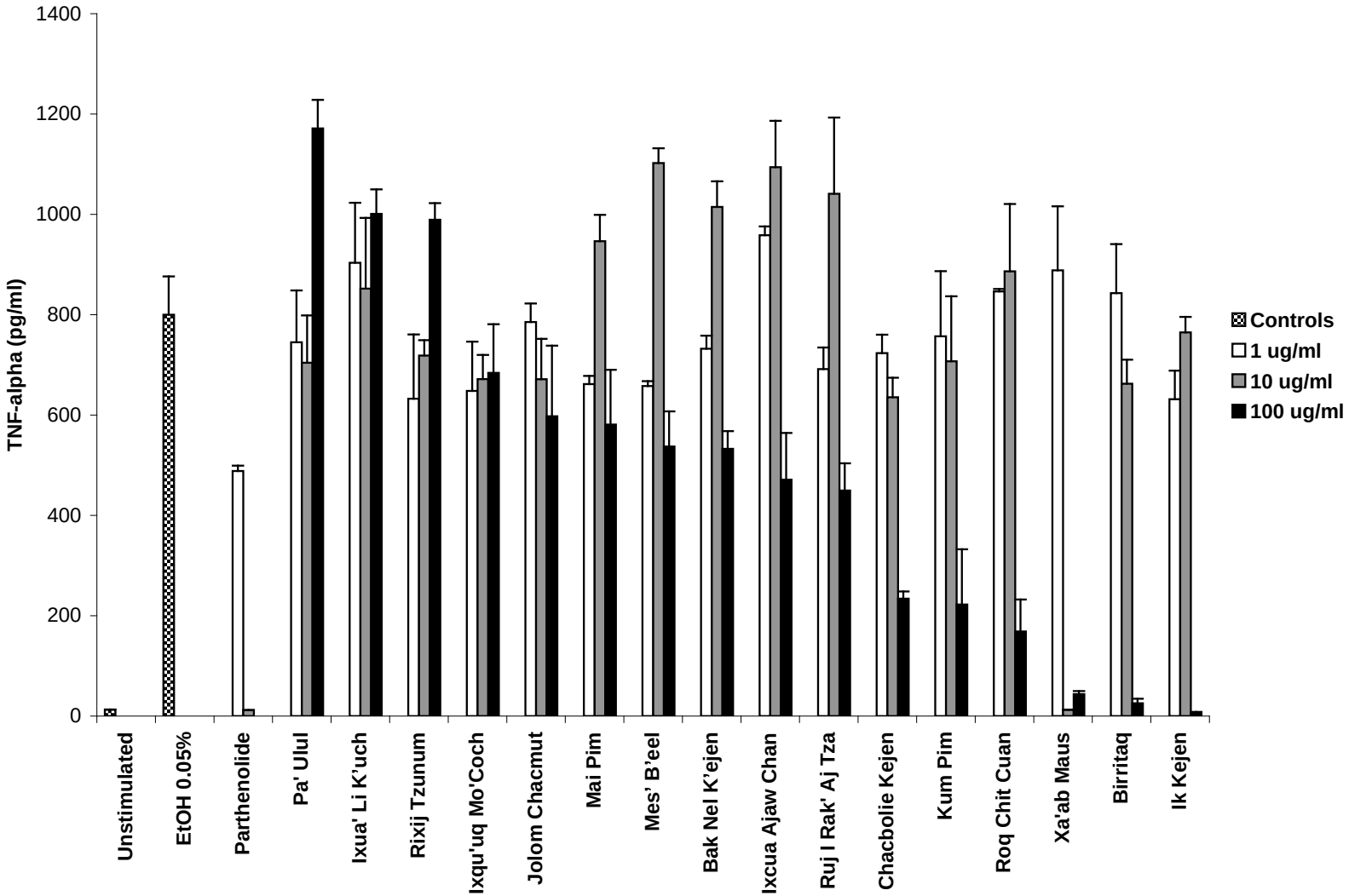

**Figure 2 -** Pro-inflammatory activity of ethanol extracts from 16 immunomodulatory plants used by the Q'eqchi Maya of Belize.

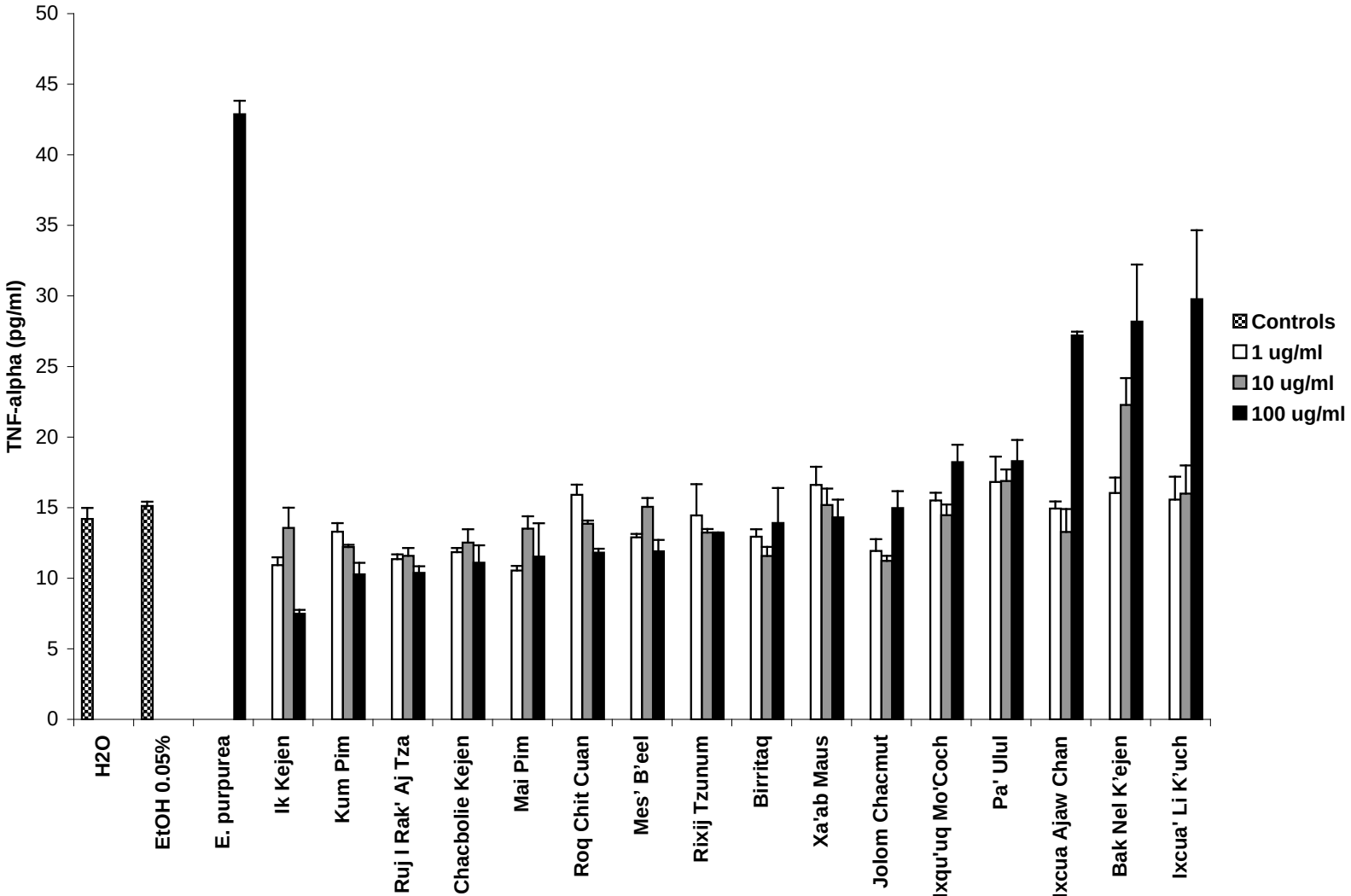

**Table 4 -** Mean zone of inhibition (mm) ± standard error of 21 ethanol extracts and controls for 6 yeast-like fungi. Zones of inhibition are represented as diameter and means (n = 3) were statistically differentiated by Tukey's test of multiple comparision.

| | *C. albicans D10* | *C. albicans Al1* | *C. albicans CN1A* | *S. cereviseae* | *C. neoformans* | *W. dermatitidis* |
|---|---|---|---|---|---|---|
| Birritaq | 0,00 $^{g}$ ± **0,00** | 0,00 $^{g}$ ± **0,00** | 10,42 ± **0,55** | 0,00 $^{g}$ ± **0,00** | 0,00 $^{e}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Mai Pim | 0,00 $^{g}$ ± **0,00** | 8,00 $^{f}$ ± **0,00** | 9,00 ± **0,29** | 8,83 $^{f}$ ± **0,30** | 0,00 $^{e}$ ± **0,00** | 9,75 $^{bc}$ ± **0,80** |
| Rixij Tzunum | 0,00 $^{g}$ ± **0,00** | 13,83 $^{d}$ ± **0,17** | 14,25 ± **0,38** | 13,83 $^{cd}$ ± **0,93** | 0,00 $^{e}$ ± **0,00** | 10,83 $^{bc}$ ± **0,33** |
| Kum Pim | 11,08 $^{c}$ ± **0,42** | 9,92 $^{ef}$ ± **0,67** | 10,08 ± **0,17** | 11,67 $^{def}$ ± **1,01** | 0,00 $^{e}$ ± **0,00** | 12,50 $^{b}$ ± **1,01** |
| Mes' B'eel | 9,58 $^{cef}$ ± **0,58** | 9,58 $^{ef}$ ± **0,71** | 9,50 ± **0,25** | 10,5 $^{ef}$ ± **0,52** | 0,00 $^{e}$ ± **0,00** | 10,67 $^{bc}$ ± **0,36** |
| Ixcua Ajaw Chan | 8,67 $^{ef}$ ± **0,44** | 8,75 $^{ef}$ ± **0,29** | 10,00 ± **0,66** | 10,17 $^{ef}$ ± **0,17** | 0,00 $^{e}$ ± **0,00** | 10,50 $^{bc}$ ± **1,32** |
| Pa' Ulul | 8,00 $^{f}$ ± **0,29** | 8,92 $^{f}$ ± **0,36** | 8,50 ± **0,00** | 0,00 $^{g}$ ± **0,00** | 0,00 $^{e}$ ± **0,00** | 8,75 $^{c}$ ± **0,43** |
| Bak Nel K'ejen | 11,00 $^{cd}$ ± **0,38** | 10,75 $^{df}$ ± **0,14** | 8,50 ± **0,14** | 9,75 $^{ef}$ ± **0,14** | 0,00 $^{e}$ ± **0,00** | 10,50 $^{bc}$ ± **0,87** |
| Chacbolie K'ejen | 9,50 $^{cef}$ ± **0,29** | 9,33 $^{ef}$ ± **0,44** | 9,58 ± **0,17** | 9,92 $^{ef}$ ± **0,60** | 0,00 $^{e}$ ± **0,00** | 9,17 $^{c}$ ± **0,44** |
| Ixcua' Li K'uch | 10,83 $^{cd}$ ± **0,96** | 11,08 $^{df}$ ± **0,36** | 10,08 ± **0,30** | 11,67 $^{def}$ ± **1,01** | 0,00 $^{e}$ ± **0,00** | 10,50 $^{bc}$ ± **0,29** |
| Jolom chacmut #1 | 11,58 $^{c}$ ± **0,33** | 13,00 $^{de}$ ± **0,38** | 0,00 ± **0,00** | 0,00 $^{g}$ ± **0,00** | 8,50 $^{d}$ ± **4,27** | 10,92 $^{bc}$ ± **0,42** |
| Xa'ab Maus | 10,67 $^{cde}$ ± **0,44** | 15,33 $^{bc}$ ± **2,03** | 0,00 ± **0,00** | 0,00 $^{g}$ ± **0,00** | 15,08 $^{c}$ ± **0,36** | 11,08 $^{bc}$ ± **0,08** |
| Ruxb'i'kaak #2 | 10,83 $^{cd}$ ± **0,55** | 12,78 $^{def}$ ± **2,58** | 8,75 ± **0,14** | 0,00 $^{g}$ ± **0,00** | 12,33 $^{cd}$ ± **0,65** | 10,25 $^{bc}$ ± **0,58** |
| Ik Kejen | 16,17 $^{b}$ ± **0,22** | 19,08 $^{ab}$ ± **0,17** | 17,50 $^{c}$ ± **0,38** | 20,25 $^{b}$ ± **0,38** | 12,08 $^{cd}$ ± **0,22** | 11,42 $^{bc}$ ± **0,36** |
| Par' I' Pim | 8,50 $^{ef}$ ± **0,29** | 13,08 $^{de}$ ± **0,68** | 12,17 $^{ef}$ ± **0,36** | 0,00 $^{g}$ ± **0,00** | 14.00 $^{cd}$ ± **0,38** | 10,42 $^{bc}$ ± **0,96** |
| Jolom chacmut #2 | 8,50 $^{ef}$ ± **0,29** | 13,17 $^{de}$ ± **0,08** | 12,00 $^{efgh}$ ± **0,80** | 0,00 $^{g}$ ± **0,00** | 16,67 $^{c}$ ± **0,55** | 0,00 $^{d}$ ± **0,00** |
| Ruxb'i'kaak #1 | 8,92 $^{def}$ ± **0,36** | 13,83 $^{de}$ ± **2,05** | 12,50 $^{def}$ ± **0,29** | 0,00 $^{g}$ ± **0,00** | 16,42 $^{c}$ ± **0,98** | 0,00 $^{d}$ ± **0,00** |
| Ruj i rak' aj tza | 9,42 $^{cef}$ ± **0,42** | 14,58 $^{cd}$ ± **1,24** | 11,50 $^{fgh}$ ± **0,29** | 12,42 $^{ce}$ ± **1,58** | 14,25 $^{v}$ ± **0,25** | 0,00 $^{d}$ ± **0,00** |
| Roq chit cuan #1 | 0,00 $^{g}$ ± **0,00** | 12,83 $^{df}$ ± **0,51** | 13,67 $^{de}$ ± **0,36** | 0,00 $^{g}$ ± **0,00** | 11,67 $^{cd}$ ± **0,74** | 0,00 $^{d}$ ± **0,00** |
| Ixqu'uq Mo'coch | 0,00 $^{g}$ ± **0,00** | 8,75 $^{f}$ ± **0,38** | 11,17 $^{fgh}$ ± **0,30** | 15,17 $^{c}$ ± **0,68** | 14,75 $^{c}$ ± **1,52** | 0,00 $^{d}$ ± **0,00** |
| Kolaras | 10,17 $^{ce}$ ± **0,22** | 0,00 $^{g}$ ± **0,00** | 10,58 $^{fgh}$ ± **0,22** | 11,58 $^{def}$ ± **1,17** | 15,83 $^{c}$ ± **0,44** | 0,00 $^{d}$ ± **0,00** |
| Ketoconazole | 26,50$^{a}$ ± **0,50** | 23,08 $^{a}$ ± **0,08** | 42,17 $^{a}$ ± **0,74** | 34.00 $^{a}$ ± **0,14** | 39.17 $^{a}$ ± **0,83** | 50 $^{a}$ ± **0** |
| Berberine | 15,50 $^{b}$ ± **0,52** | 20,08 $^{ab}$ ± **0,33** | 23,00 $^{b}$ ± **0,38** | 20,58 $^{b}$ ± **0,55** | 25,83 $^{b}$ ± **0,87** | 50 $^{a}$ ± **0** |
| 80 % Ethanol | 0,00 $^{g}$ ± **0,00** | 0,00 $^{g}$ ± **0,00** | 9,08 ± **0,08** | 0,00 $^{g}$ ± **0,00** | 0,00 $^{e}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |

**Table 5 -** Mean zone of inhibition (mm) ± standard error of 21 ethanol extracts and controls for 3 gram-positive and 3 gram-negative bacteria. Zones of inhibition are represented as diameter and means (n = 3) were statistically differentiated by Tukey's test of multiple comparision.

| | **Gram-positive** | | | **Gram-negative** | | |
|---|---|---|---|---|---|---|
| | *B. subtilis* | *E. faecalis* | *L. innocua* | *E. coli* | *P. putida* | *P. stuartii* |
| Birritaq | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Mai Pim | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Rixij Tzunum | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Kum Pim | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Mes' B'eel | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8.33 $^{c}$ ± **0,17** | 0,00 $^{d}$ ± **0,00** |
| Ixcua Ajaw Chan | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Pa' Ulul | 0,00 $^{b}$ ± **0,00** | 0,00 $^{f}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8.33 $^{c}$ ± **0,17** | 0,00 $^{d}$ ± **0,00** |
| Bak Nel K'ejen | 0,00 $^{b}$ ± **0,00** | 10,75 $^{c}$ ± **0,25** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8.33 $^{c}$ ± **0,17** | 0,00 $^{d}$ ± **0,00** |
| Chacbolie K'ejen | 0,00 $^{b}$ ± **0,00** | 9,08 $^{de}$ ± **0,17** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Ixcua' Li K'uch | 0,00 $^{b}$ ± **0,00** | 9,58 $^{cd}$ ± **0,36** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Jolom chacmut #1 | 0,00 $^{b}$ ± **0,00** | 8,75 $^{de}$ ± **0,14** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 8,00 $^{c}$ ± **0,00** |
| Xa'ab Maus | 0,00 $^{b}$ ± **0,00** | 8,50 $^{de}$ ± **0,25** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 9,00 $^{ba}$ ± **0,14** |
| Ruxb'i'kaak #2 | 0,00 $^{b}$ ± **0,00** | 9,25 $^{de}$ ± **0,25** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 8,17 $^{bc}$ ± **0,17** |
| Ik Kejen | 0,00 $^{b}$ ± **0,00** | 9,33 $^{de}$ ± **0,46** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 9,17 $^{a}$ ± **0,22** |
| Par' I' Pim | 0,00 $^{b}$ ± **0,00** | 9,17 $^{de}$ ± **0,22** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |
| Jolom chacmut #2 | 0,00 $^{b}$ ± **0,00** | 8,50 $^{de}$ ± **0,50** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 8,33 $^{bc}$ ± **0,08** |
| Ruxb'i'kaak #1 | 0,00 $^{b}$ ± **0,00** | 8,92 $^{de}$ ± **0,08** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 8,42 $^{ac}$ ± **0,22** |
| Ruj i rak' aj tza | 0,00 $^{b}$ ± **0,00** | 9,08 $^{de}$ ± **0,30** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 9,25 $^{a}$ ± **0,43** |
| Roq chit cuan #1 | 0,00 $^{b}$ ± **0,00** | 8,92 $^{de}$ ± **0,08** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** | 8,58 $^{ca}$ ± **0,46** |
| Ixqu'uq Mo'coch | 0,00 $^{b}$ ± **0,00** | 9,08 $^{de}$ ± **0,17** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8.33 $^{c}$ ± **0,17** | 0,00 $^{d}$ ± **0,00** |
| Kolaras | 0,00 $^{b}$ ± **0,00** | 8,33 $^{e}$ ± **0,17** | 0,00 $^{c}$ ± **0,00** | 0,00 $^{c}$ ± **0,00** | 8.00 $^{c}$ ± **0,00** | 0,00 $^{d}$ ± **0,00** |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ampicillin | 0,00 [b] ± **0,00** | 22,33 [a] ± **0,08** | 24,50 [a] ± **1,23** | 9,42 [b] ± **0,17** | 19,42 [a] ± **1,21** | 0,00 [d] ± **0,00** |
| 80 % Ethanol | 0,00 [b] ± **0,00** | 0,00 [f] ± **0,00** | 0,00 [c] ± **0,00** | 0,00 [c] ± **0,00** | 0,00 [d] ± **0,00** | 0,00 [d] ± **0,00** |