# BIOACTIVITY OF AND PHYTOCHEMICAL STUDIES ON EXTRACTIVES FROM SOME GHANAIAN PLANTS

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**ABSTRACT** - Ten indigenous plants with folkloric reputation for pesticidal activity have been screened for their biological activity using the brine shrimp lethality test. Analyses were carried out on leaves and bark of each plant. The leaves of *Annona squamosa* and *Piper guineense* and the bark of *Carapa procera* and *Piper guineense* with $LC_{50}$ less than 25 ppm were very active. All extracts which gave $LC_{50}$ values of less than 200 ppm were considered bioactive and were screened for their phytochemicals using solvents of different polarities.

**Keywords - Bioactivity, bioassay, extractives, phytochemicals**

## INTRODUCTION

Plants are vast resources of secondary metabolites. The role that some secondary metabolites from plants play in pest management is well recognized (Bowers, 1983 Jacobson, 1977). A wide array of phytochemicals ranging from simple molecules like oxalic acid to complex molecules like cyanogenic glycosides, alkaloids, lipids, terpenoids, saponins, flavonoids, tannins and lignins have been found to mediate plant-herbivore-natural enemies interaction. Isolation of bioactive natural products from plants can be quite cumbersome if inexpensive and simple "bench-top" bioassays are not available. The brine shrimp lethality test is a quick, inexpensive and practical method of testing the bioactivity of natural products. It is particularly useful in determining the cytotoxicity, pharmacologic actions and pesticidal effects of natural products (McLaughlin <u>et al</u>, 1991; McLaughlin,1991)

Irvine (1961) listed at least 50 plants from Ghana which are used traditionally in various forms or preparations as pesticides. The pesticidal effects of some of these plants have been demonstrated (Cobbinah & Appiah-Kwarteng 1990; Cobbinah & Tuani, 1992). In this paper we report the relative bioactivities of the ethanol and petroleum ether extractives of 10 indigenous plants.

## MATERIALS AND METHODS

The plants were collected from the botanical gardens of the University of Science and Technology, Kumasi, and identified at the herbarium of the Forestry Research Institute of Ghana in Kumasi. *Artemia salina* (brine shrimp eggs) and artificial sea water were supplied by J.L. McLaughlin of School of Pharmacy and Pharmaceutical Studies, Purdue University, West Lafayette, USA.

### Extraction

The leaves and bark of each plant were sun dried, powdered in a roller mill and exhaustively extracted successively with petroleum ether (40-60°C) and ethanol in a soxhlet extractor.

### Bioassay

Brine shrimp eggs were hatched in artificial sea water and used after 48h. Methanolic formulations of plant extracts were prepared in vials at concentrations of 1000ppm, 100 ppm and 10 ppm. The extracts were freed of solvent by placing them in fume hood overnight. The alcoholic and petroleum ether extracts were subsequently dissolved and dispersed respectively in

**Table 1a. Comparative toxicity of ethanol extracts of 10 indigenous plants to the brine shrimp (*Artemia salina*)**

| Botanical name | Plant part | $LC_{50}$(ppm) | 95% CL |
|---|---|---|---|
| *Acacia hockii* | Leaves | 110.9 | 53-237 |
| | Bark | 40.6 | 20- 72 |
| *Adansonia digitata* | Leaves | >1000 | - |
| | Bark | 81.5 | 45-142 |
| *Annona squamosa* | Leaves | 16.2 | 7- 28 |
| | Bark | 240.7 | 131-480 |
| *Anogeissus leiocarpus* | Leaves | >1000 | - |
| | Bark | 307.8 | 189-153 |
| *Carapa procera* | Leaves | 73.8 | 44.119 |
| | Bark | 7.4 | 1- 18 |
| *Datura innoxia* | Leaves | - | - |
| | Bark | >1000 | - |
| *Erythrophleum guineense* | Leaves | >1000 | - |
| | Bark | 43.4 | 21-79 |
| *Pentadesma butyracea* | Leaves | 160.4 | 55-639 |
| | Bark | 325.8 | 104-357 |
| *Piper guineense* | Leaves | 32.9 | 14-62 |
| | Bark | 30.4 | 24-225 |
| *Tabernaemontana crassa* | Leaves | 251.4 | 107-865 |
| | Bark | 66.2 | 22-165 |

5ml of artificial sea water. Ten brine shrimps were introduced into each of the vials via a dipso-pipette (three vials were set up for each concentration and a control check). The vials were then placed in a holding room at 25 ± 3° C with a relative humidity 80 ± 5% for 24h. Mortality was recorded after 24h and $LC_{50}$ values determined according to SAS (1985).

**Phytochemical Screening**

Solvent extracts showing high levels of bioactivity were screened for the various phytochemicals using the approaches suggested by Das and Bhattacharjee (1972) and Feigl (1960).

Data from all tests were corrected for control mortality using Abbots formula (1925). $LC_{50}$ values were considered to be significantly different only when 95% confidence intervals did not overlap.

## RESULTS AND DISCUSSION

The toxicities of the ethanol and petroleum extracts of the 10 plant species are given in tables 1a and 1b. With the exception of West African Pepper *(Piper guineense)* (table 1a) and *Tabernamontana crassa* (table 1b) differences in $LC_{50}$ between leaves and bark were large and may be due to qualitative and quantitative differences of active ingredients in one of the two plant parts. The West African pepper was the only plant species which showed high levels of bioactivity in both the ethanolic and petroleum-ether extract from the leaves and bark of the two solvents used. The fiducial limits of the $LC_{50}$ values for the leaves and bark for the two solvent system overlapped indicating no difference in activity of the four extracts.

In contrast, the bioactivity of the leaves and bark of the Sugar apple

**Table 1b: Comparative toxicity of Petroleum ether extracts of 10 indigenous plants to the brine shrimp (*Artemia salina*)**

| Botanical name | Plant part | $LC_{50}$(ppm) | 95%CL |
|---|---|---|---|
| *Acacia hockii* | Leaves | >1000 | - |
| | Bark | 249.6 | 92-1242 |
| *Adansonia digitata* | Leaves | >1000 | - |
| | Bark | - | - |
| *Annona squamosa* | Leaves | 8.2 | 2-14 |
| | Bark | 133.1 | 73-245 |
| *Anogeissus leiocarpus* | Leaves | >1000 | - |
| | Bark | >1000 | - |
| *Carapa procera* | Leaves | 105.2 | 54-204 |
| | Bark | >1000 | - |
| *Datura innoxia* | Leaves | - | - |
| | Bark | 225.9 | 117-490 |
| *Erythrophleum guineense* | Leaves | >1000 | - |
| | Bark | 248.2 | 130-534 |
| *Pentadesima butyracea* | Leaves | >1000 | - |
| | Bark | >1000 | - |
| *Piper guineese* | Leaves | 15.8 | 1-44 |
| | Bark | 20.9 | 7-41 |
| *Tabernaemontana crassa* | Leaves | 467.6 | 134-879 |
| | Bark | 415.9 | 132-5989 |

(*Annona squamosa*),another promising plant species for development of botanicals (Grainge Ahmed, 1987) differed significantly in both solvents. The $LC_{50}$ values for the bark were 15 and 17-folds higher than in the leaves for the ethanolic and petroleum-ether extracts respectively.

Combining results of tables 1a and 1b and considering the most active extract thereof the 10 plant species can be arranged in decreasing order of toxicity based on $LC_{50}$ values as *Carapa procera* (7.4ppm) > *Annona squamosa* (8.2ppm) > *Piper guineense* (15.8ppm) > *Acacia hockii* (40.6 ppm) > *Erythrophleum guineense* (43.4ppm) > *Tabernaemontana crassa* (66.2 ppm) > *Adansonia digitata* (81.5 ppm) > *Pentadesma butyracea* (160 ppm) > *Datura innoxia* (225.9 ppm) > *Anogeissus leiocarpus* (307.8 ppm). (*Carapa procera*), the sugar apple (*Annona squamosa*) and the West African pepper with very low $LC_{50}$s were the most toxic in our studies. *Anogeissus leiocarpus* was the least toxic plant tested. However, the $LC_{50}$ of 307.8 ppm recorded for the ethanol extract of the bark indicates a moderate level of bioactivity. It is possible that extracts that showed least activity in the 24h bioassay will be effective at longer exposure time. In such cases in vivo activation may be necessary. Rose and Sparks (1984) observed that Acephate require much longer exposure period to produce mortality compared to other organo- phosphates.

Grainge and Ahmed (1987) have listed all 10 plants tested as plants with folkloric pest control properties. Our results indicate that there may be sufficient scientific backing for the folk-loric pesticidal activity ascribed to some of the indigenous plants. Preliminary phytochemical screening of ethanolic and petroleum-ether extracts of both the leaves and

**Table 2: Major phytochemical substances in the 10 tests plants**

| Plant Name | Plant Part | Major phytochemical substances A | C | F | G | P | S | T |
|---|---|---|---|---|---|---|---|---|
| *A. hockii* | leaves | | | x | x | x | x | x |
| *A. hockii* | bark | | | x | x | x | x | x |
| *A. digitata* | bark | | | x | x | | | x |
| *A. squamosa* | leaves | x | x | | x | x | x | x |
| *C. procera* | leaves | x | x | x | x | | x | x |
| *C. procera* | bark | x | x | x | x | | x | x |
| *E. guineense* | bark | x | x | x | x | | x | x |
| *P. butyraceae* | leaves | | | x | | x | x | x |
| *P. guineense* | leaves | x | | | x | | x | x |
| *P. guineense* | bark | x | | | | | x | x |
| *T. crassa* | bark | x | | | x | | x | x |

**A - Alkaloids, C - Coumarins, F - Flavonoids,**
**G - General glycosides S - Saponins, T - Tannins**
**P - Terpenoids/Steroids**

bark of the 10 plant species showed at least 5 different classes of secondary plant substances.
Alkaloids and tannins which were found in all the 10 plant species have been shown to be responsible for the bioactivity of *Annona squamosa*, *Piper guineense*, *Adansonia digitata* and related species of *Acacia hockii*, *Erythrophleum guineense*, and *Tabernaemontana crassa* (Grainge & Ahmed 1987). The bioassay used in our study is a quick way of determining the bioactivity of natural products. Using the brine shrimp lethality test for bioactivity guided screening and fractionation McLaughlin (1991) has been able to rapidly detect and isolate a variety of novel chemically bioactive compounds. A field test to determine the effects of the extracts on a range of pests is suggested. The feasibility of the use of these materials can be assessed after extensive field trials. Undoubtedly, plant derived toxicants are invaluable sources of potential insecticides and would enhance current and future chemical control of pests particularly in developing countries where these plants abound.

**ACKNOWLEDGEMENT** - The project was supported in part by the International Foundation for Science through grant F/982-2 to J.R. Çobbinah. We thank Ms. Augustina Addai of Forestry Research Institute of Ghana for identification of plant species.

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