SCREENING OF *Gmelina arborea* (Yemane) LEAF EXTRACTS FOR ANTIMICROBIAL, PHYTOCHEMICAL AND PESTICIDAL PROPERTIES

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ABSTRACT

Leaves of *Gmelinaarborea*were extracted using ethanol, methanol, and distilled water. Preliminary phytochemical screening of the extract revealed the presence of fats and oils, hydrolysable tannins, 2-deoxysugars, saponins and tannins. The agar disc diffusion method was used to determine the antibacterial and antifungal activity on three Gram (+) bacteria (*B. cereus, B. subtilis, and S. aureus*), two Gram (-) bacteria (*E. coli and S. typhi*) and a fungus (*C. albicans).* The highest activity was shown by the aqueous extract with a mean diameter of inhibition zone ranged from 15.33 – 24.67 mm followed by ethanol which had 14 – 23.67 mm and lastly was observed in methanol which had 14-22.33 mm. The minimum inhibitory concentration (MIC) of the extracts for *E. coli, S. typhi* and *C. albicans* was 56 mg/mL, for *B. cereus, B. subtilis, and S. aureus* ranged from 14 mg/mL – 28 mg/mL. Pesticidal activity screening was determined using a *Brassica juncea* (mustasa) and lethal dosage was assessed using sawfly *(Athalia lugens proxima).* Lethal dosage was carried out using four concentrations (70%, 80%, 90% and 100%) for each of the extracts, which involved the determination of time of paralysis and time of death of the worm. The best activity was found in aqueous extract having 90% mortality rate in just 18 seconds, followed by ethanol and methanol both having 80% mortality rate in 25 and 29 seconds respectively. These promising findings suggest the presence of antibacterial and antifungal agents in the tested plant material, exhibited by its bioactive compounds, and serving them as an alternative antimicrobial agent against the tested microorganisms, furthermore, alcoholic and aqueous extract significantly demonstrated death of sawfly especially at higher concentration of the extract making this plant a potential agent as an organic pesticide.

Keywords: sawfly*, Brassica juncea* (mustasa), phytochemical screening

1 INTRODUCTION

Plants are used by man in a variety of ways. Some are used for landscaping and ornamentation; it has been the extraordinary source for the discovery of new products of medicinal value for drug development. Aside from they are rich in nutrients; they are also rich in compounds which have pain relieving and healing abilities. The abundance of compounds, found in plants, made plant-based traditional medicine system to continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Doughari et. al. 2008).

For the past few years, population has increased at a tremendous rate, hence prohibitive cost of treatments have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Worldwide, infectious diseases are the number one cause of death accounting for approximately one-half of all

deaths in tropical countries (Iwu et. al. 1999).

Natural plants have been seen as a valuable source of microbial agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents (Valarmathy et. al. 2010). The used of plant extracts with known antimicrobial properties can be of great significance in the therapeutic treatments. Among the estimated 250,000 – 500,000 plant species, only a small percentage has been investigated phytochemically, and the fraction submitted to biological or pharmacological screening is even smaller (Mahesh & Satish 2008).

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant (Bishnu et. al. 2009). Medicinal plants are rich in a numerous variety of secondary metabolites of antimicrobial properties such as saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Abdallah 2011). There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them (Peteros & Uy 2010).

Plants are also used in botanical pesticides. Insect pests have been one of man's most serious problems because they increase in number so fast, they cause diseases such as H-fever, malaria, dengue, filariasis, etc. and they destroy crops. An effective pest control is no longer a matter of heavy application of pesticides, partly because of the rising cost of petroleum derived products but, largely because excessive pesticide use promotes speedier evolution of resistance in insect pests, destroys natural enemies, turns formerly harmless species into pests, harms other non-target species, and contaminates food.

Another problem with the continued reliance on chemical pesticide is its capacity to cause pest build-up. One documented case is that on the diamond black moth (*Plutella xylostella, L.*) which exhibits multiple resistances to malathion, methyl parathion, DDT, diazonin, meviaphos, and carbyl, and is developing resistance to newly introduced insecticides. The pesticidal formulations based on herbal products have attracted particular attention because of their specificity to insect pests, their biodegradable nature and their potential for commercial application.

With these aforementioned reasons, the researchers investigated the "yemane", scientifically known as *Gmelina arborea,* a medium-sized deciduous tree up to 40m tall and 140 cm in diameter, but usually smaller than this. Ethnobotanical studies reported that the species is widely used to treat many diseases including diarrhoea, hypertension and malaria, among others. This paper reported the antimicrobial and pesticidal properties of yemane (*Gmelina arborea*). Pesticides that will be made out from this plant are environment-friendly and cheap.

2 MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The fresh leaves of *Gmelina arborea* were collected from Angono, Rizal, Philippines. The leaves were washed with water to remove dirt and unwanted particles. The samples were air-dried for one week at room temperature and then, pounded separately using mortar and pestle into smaller particles, and later reduced to powder using electric blender. The powder samples were stored in airtight containers and kept at room temperature until required.

2.2 Microorganisms and Culture Media

The test microorganisms were obtained from Microbiology and Genetics Division, ITDI-DOST. Five bacterial strains and a fungus known to be pathogenic were used in the experiment. These included three Gram (+) bacteria (*Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus*), two Gram (–) bacteria (*Escherichia coli, Salmonella typhi)* and a

fungus *Candida albicans.* The bacterial strains were grown and sub cultured on Nutrient Agar while the fungus was grown on Potato Dextrose Agar. The cell suspension was prepared at 1.0 x 10^7 colony forming units (CFU/mL) following McFarland 0.5 turbidity standard.

2.3 Extraction Procedure

The air-dried powdered plant samples (25 g) were soaked separately in 250 mL of 70 % ethanol (70:30), 60 % methanol (60:40) and distilled water in a 500 mL sterile flask for 48 h at ambient temperature (35 ºC), and shaken occasionally. The extracts were filtered using a cheese cloth and later filtered again using a Whatman filter paper no. 1 (El-Mahmood et. al. 2010). Each of the filtrate was evaporated in water bath from 30 minutes to 1 hour to evaporate the alcohol; and the extracts acquired were stored in airtight flask until required.

2.4 Phytochemical Screening

The 150 g of powdered plant samples were submitted to the Chemical and Energy Division of ITDI-DOST for the preliminary phytochemical screening of *G. arborea* to identify and characterize some of its composition. A guide book to Plant Screening-Phytochemical & Biological, Revised Edition 2005 was used and adopted.

2.5 Antimicrobial and Antifungal Screening

The screening for antibacterial activity of the extract was performed using three Gram (+) bacteria (*S. aureus, B. subtilis* and *B. cereus*) and Gram (-) bacteria (*S. typhi* and *E.coli*). The antifungal property of the extracts was also determined using *Candida albicans.*

Agar Disc Diffusion assay was carried out for determination of antimicrobial activity of the extracts. Briefly, 5 mL of 18 h test culture adjusted to 0.5 McFarland was used as an inoculum. The inoculum was then added to a plated agar; spread plate method was used using L- shaped rod. After which, the inoculum was incubated until a moisten surface was achieved. Then, Whatman filter paper no. 1 discs (6 mm in diameter) were soaked in plant extract (aqueous, methanol and ethanol extracts) of *G. arborea* to completely saturate the discs, and then placed on bacterial culture-seeded plates. Each sample was performed in triplicates to determine the consistency of the possible antibacterial and antifungal activity. The procedure was repeated for all of the organisms used. The inoculated petri dishes were placed at room temperature and incubated at 35ºC for 24 hours. The antibiotic streptomycin sulphate (10 mg/mL) was used as a positive control in bacteria and ketokonazole (1mg/mL) for the fungus. Each extract was assayed in triplicate. Antimicrobial efficacy of the extracts was evaluated by measuring the diameter of zone of inhibition in millimetres.

2.6 Minimum Inhibitory Concentration (MIC)

Microbroth dilution method with slight modifications was followed for determination of minimum inhibitory concentration (MIC). Extracts of *G. arborea* were suspended in a series of tubes containing 5 mL nutrient broth. The first tube containing a 5 mL of nutrient broth was added by 7 mg of extract to have a final concentration of 7 mg/mL; consequently 14 mg of extracts were diluted to 5 mL of nutrient broth to have a concentration of 14 mg/mL. The procedure was repeated until a concentration of 448 mg/mL was achieved. The inoculum was prepared in 0.1 % peptone from the isolated bacterial colonies selected from 18 to 24 hours cultured organisms. Suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard, each tube should contain approximately 1.0 x 107 colony forming units (CFU/mL). Within 15 minutes after the inoculum has been standardized, a loopful of the adjusted inoculum was added to each tube containing 5 mL plant extract and then mixed well to distribute the microbial cells uniformly. The tubes were then incubated for 24 hours. A tube containing an extracts without organisms from different solvents were used as a negative control to determine if there was a change in turbidity with

the extract with organisms. The tubes were examined for the change in turbidity by comparing the negative control to that of the tubes with inoculated microorganisms. The reading was also compared to McFarland standards to know the exact turbidity of the extract after the incubation period.

2.7 Pesticidal Activity Screening

The extracts of *G. arborea* were evaluated for its possible pesticidal property. Two-week old *Brassica juncea* (mustasa) which were pesticide-free, were purchased at Bureau of Plant Industry in Quezon City, Philippines. The plants were divided into five groups for aqueous, ethanol, methanol, control (treated by malathion 1 tbsp/10 mL) and untreated. To evaluate if the extracts were effective in preventing the occurrence of pests in the *Brassica juncea,* the extracts were applied in different parts of the plant. Four different concentrations were used in the study mainly, 70%, 80%, 90%, and pure extracts.The application of extracts and the malathion (control) were done through spraying in the leaves of *Brassica juncea*. The extracts and control were applied in the 3rd week of the plant; for it's the time when the pests occur. The application of the extracts and the malathion were assessed; in every day spraying, then every other day and once a week.

The effectiveness of the extract to kill the sawfly *(Athalia lugens proxima)* present in the mustasa plant was also evaluated. The time of death after one spraying to a group of pests were also recorded. The lethal dosage of the *G. arborea* leaf extracts was also determined by spraying the extracts directly to the sawfly and timed until death.

3 RESULTS AND DISCUSSIONS

3.1 Phytochemical Screening

Phytochemical screening of the extract of *G. arborea* revealed the presence of fats and oils, hydrolysable tannin, saponins, 2-deoxysugars and free fatty acids (Table 1). These compounds have significant application against human pathogens, including those that cause enteric infections.

Table 1: Phytochemical analysis of *Gmelina aborea* (yemane) leaf extract

QUALITATIVE TESTS	RESULTS	INDICATIONS
Filter Paper Test	Greasy appearance observed after drying of filter paper	Presence of fats and oils
Dragendroff's Test	No reaction (-)	Absence of alkaloids
Mayer's Test	No reaction (-)	Absence of alkaloids
Ferric Chloride Test	Blue-black color (+)	Presence of hydrolysable tannin
Froth Test	Froth formed more than 2cm (+)	Presence of saponins
Keller Kiliani Test	Reddish-brown color which turned purple (+)	Presence of 2-deoxysugars
Liebermann-Burchard Test	No reaction (-)	Absence of unsaturated steroids and triterpenes
Sodium Carbonate Test	Formed stable and dense froth (+)	Presence of free fatty acids
Bate-Smith & Metcalf Test	No reaction (-)	Absence of leucoanthocynins
Wilstatter 'Cyanidin' Test	No reaction (-)	Absence of µ-benzopyrone nucleus
Bomtrager's Test	No reaction (-)	Absence of anthraquinones

Legend: (+) = presence of compound and (-) = absence of compound

Ferric chloride test in the *G. arborea* leaf extract showed a dark colored precipitates that indicated the presence of tannins. Recent report shows that tannins have potential medicinal value. They could be used as a treatment for diarrhea and extensive burns and maybe used for the relief of various rectal disorder and excretion. They can also be used in the treatment of bedsore and weeping ulcers. These tannins were also formerly used for sore throat and stomatitis (Banez & Castor 2011). This suggests that the plant could be potential source of treatment of the above-mentioned diseases. Phytochemical analysis revealed the presence of fats and oils in the leaf extract of *G. arborea.* The fats and oils belong to lipid biomolecules. Lipids are a class of hydrocarbon-containing organic compounds essential for the structure and function of living cells.

Essential oils have been used extensively in ancient Rome, Greece, Egypt & the Middle East – as perfumes, flavours, deodorants, antiseptics & pharmaceuticals. As a result of new processing technologies, they can today be used for more applications as well (Banez & Castor 2011). The froth test showed that upon testing the *G. arborea* extract, a froth with a size of 2 cm was formed that indicated a presence of saponins. Recent research showed the potential of a naturally occurring phytosterol found in certain plants called "saponins". These saponins work as the plant's natural immune system; a natural antibiotic that protects the plant against harmful microbes and fungus. In humans, these same saponins, with no adverse side effects, are a "natural bile acid sequestrant" that lowers cholesterol, strengthens the immune system and fights off pathogens that can cause chronic arthritic pain and low grade inflammation. Saponins work entirely within the digestive system, binding with cholesterol from the liver bile and dietary cholesterol, along with intestinal pathogens, making them unavailable for re-absorption. This mixture isthen removed from the body through the normal elimination process.

Cardiac glycosides result was confirmed by the occurrence of reddish-brown color which turned purple in the Keller Kiliani test. Cardiac glycosides are secondary metabolites created by plants and animals. They are usually toxic but may have drug-like therapeutic effects when used appropriately. Cardiac glycosides are complex triterpene molecules, created by plants and amphibians that exert intense biological effects in humans and many other organisms. While extremely toxic, these molecules often have therapeutic use when dosed appropriately in minute quantities. In the case of free fatty acids, it was confirmed by the presence of stable and dense froth. Free fatty acids have potent antimicrobial, antiviral and antifungal properties, and they exert such effects in some living systems, especially the skin and mucosa of the lung. Free fatty acids are effective also in operating at specific intracellular locations reversibly to amplify or otherwise modify signals. The presence of the identified phytochemicals makes the leaves pharmacologically active (Syed et al. 2011). Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases.

3.2 Antimicrobial and Antifungal Screening

The antibacterial activity of *G. arborea* extract against pathogenic microorganisms showed a considerable success. Antimicrobial screening by agar disc diffusion method has shown that all extracts of leaves of *Gmelina arborea* has an antibacterial and antifungal activity against all microbial strains (Table 2).

Table 2: Antimicrobial and antifungal activity of *G. arborea* extracts against the test organisms

Organisms	**Aqueous**	**Ethanol**	**Methanol**	**Control**
	Mean of Zone of Inhibition (mm)	Mean of Zone of Inhibition (mm)	Mean of Zone of Inhibition (mm)	Mean of Zone of Inhibition (mm)
B. cereus	24.33	22.67	22.33	35
B. subltilis	19.33	16.33	15.67	27.67
S. aureus	24.67	23.67	22	31.33
E. coli	19	18.33	14.67	29.33
S. typhi	19	18.33	17	26.33
C. albicans	15.33	14	14	25.67

The results that were presented here are relevant, as the literature has shown that Gram (+) bacteria are more sensitive to antibiotics. The Gram (-) bacteria display some particularities that inhibit antibiotics penetration, as the lipopolysaccharide layer that determines the permeability and susceptibility to antibiotics. The data obtained here may suffer seasonal influence and/or be associated with the presence of chemical compounds derived from *G. arborea* species secondary metabolism as reported in phytochemical studies. In the previous study by Ambujakshi et. al.(2009) using extracts from *G. arborea* species showed that they were able to inhibit the growth of Gram (-) bacteria strains. However, in this study, the antibacterialactivity against Gram (-) bacteria was verified, mainly *B. cereus, B. subtilis and Staphylococcus aureus*. The antimicrobial activity of the plant was further tested because of their great medicinal relevance with the recent years; infections have increased to a great extent and resistant against antibiotics become an ever increasing therapeutic problem. The results obtained are encouraging as the aqueous, ethanol, and methanol extracts have shown considerable antibacterial and antifungal activity against the tested organisms.

The extracts presented antibacterial and antifungal activities against clinically relevant pathogens (gram positive, gram negative and yeast). Though all extract were found effective against bacteria and yeast, aqueous extracts showed maximum inhibition against the test organisms. The least activities were recorded in methanol extract that ranged from 14 to 22.33 mm diameter of zone of inhibition. The results of the antibacterial and antifungal of the extracts were however less than the conventional antibiotic streptomycin (26.33 to 35 mm) and antifungal ketoconazole (25.67 mm) maximum diameter of zone of inhibition.

3.3 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC), which is the smallest concentration of *G. arborea* extract that was capable of inhibiting the growth of the tested microorganisms, was assessed.After 24 hours of incubation, the extracts showed a varying degree of differences in inhibiting the growth of organisms.

The MIC values demonstrated that the extracts showed different levels of inhibitory effects depending on its concentration. The results of the present study indicated that the aqueous extracts exhibited the strongest activity against all organisms used.

Table 3. The minimum inhibitory concentration of aqueous and alcoholextract of *G. arborea.*

Organisms	MIC of extracts (mg/mL)		
	Aqueous	**Ethanol**	**Methanol**
B. cereus	14	14	28
B. subltilis	14	28	28
S. aureus	14	14	28
E. coli	56	56	56
S. typhi	56	56	56
C. albicans	56	56	56

The results indicated that the aqueous extract had the best results in terms of inhibiting the growth of the tested organisms, followed by the ethanol extract and last was methanol. Of all the organisms used in the determination of MIC, *E. Coli, S. Typhi,* and *C. Albicans* showed to be the least sensitive to aqueous, ethanol and methanol extracts consistently having 56 mg/mL MIC values.

The results gathered in MIC evaluation supported the agar disc diffusion assay results, claiming that the aqueous extracts were the most effective among the extracts used by having the highest zone of inhibition ranged from 15.33 – 24.67 mm and the lowest MIC values of 14 mg/mL for the three gram positive bacteria mainly (*B. Cereus, B. Subtilis, and S. Aureus)* and 56 mg/mL in *E.coli, S. Typhi and C. Albicans.* The highest MIC values recorded in *E.coli, S. Typhi and C. Albicans* is an indication that either the plant extracts are less effective on some gram negative bacteria and yeast or that the organism has the potential of developing resistance against the bioactive components of the extracts, while the low MIC values for other bacteria is an indication of the efficacy of the plant extracts.

3.4 Pesticidal Screening

The results of the pesticidal activity screening of the aqueous and alcoholic extracts against sawfly *(Athalia lugens proxima)* are presented in Table 4 (Data not shown). The percentage mortality was found to increase with the corresponding increase in dosage, indicating a direct relationship between the two.

Among the extracts used in the pesticidal screening study of the extracts of *G. arborea,* the aqueous extracts showed the greatest impact compared to the remaining two extracts against the saw fly (*Athalia lugens proxima)* by having 90% mortality rate in LD100 in just 18 seconds. The highest value by LD100 was followed by a 70% mortality rate in LD90 and 30% mortality rate in LD80 in just 33 and 42 seconds respectively; the least performance was however observed in LD70 having no impact against the sawfly.

In case of the ethanol extracts; it followed the same trend as with the aqueous extracts. LD100 showed 80% mortality rate in a time of 25 seconds, followed by 40% mortality rate in LD90 in just 40 seconds and lastly is the LD80 having a 10% mortality rate in 40 seconds. The LD70 however showed no effect against the tested sawfly.

The methanol extract showed the least activities compared to ethanol and aqueous extracts. The LD100 showed an 80% mortality rate in 29 seconds after the application of extracts, followed by LD90 having amortality rate of 30% in a span of 39 seconds. The LD80 and LD70 were found to be ineffective against the tested sawfly.The application of extracts were also assessed and the results showed that in the first week after spraying the test plant showed no symptoms of being infected by the saw fly. However the untreated, which plants were not sprayed by any extracts showed that there was a present of sawfly larvae. Furthermore, in the final week of observation the treated plants were consistently grown healthy and free of infections, while the untreated showed a severe infection caused by the sawfly.

The control malathion was however showed a stellar performance by killing the sawfly in as low as LD70 in 40 seconds. LD80 showed a 70% mortality rate in just 21 seconds, LD90 with 90% mortality rate and LD100 with 100% mortality rate in just 15 and 13 seconds respectively.Data gathered with these extracts indicated that the aqueous extracts remained to be the most effective having 90% mortality rate in LD100 in just 18 seconds compared to ethanol and methanol both having 80% mortality rate in 25 and 29 seconds, respectively.
In terms of determining the lethal dosage of the extracts, aqueous extract was found to be effective pesticide agent in as low as 80% concentration (80:20 extract:water), while ethanol was found to beeffective in as low of 80% concentration (80:20 extract:water) but the effect is so minimal; only killing 10% of the treated sawfly. Methanol having the least efficacy among the extracts used had a lethal dosage at 90% (90:10 extract:water) concentration.

This study clearly showed that the pests' population declined drastically relative to concentration and type of extracts used. It is evidently showed that the extracts of *G. arborea* can be made into an effective pesticide. The aqueous extract has almost the same efficacy as that of malathion making this a remarkable success.

This present research can however relate to the study of Ambujakshi et al. (2009) reported that *G. arborea* alcoholic and aqueous extracts *also* exhibited antihelminthic activity against *Ascardia galli* worms giving short time of paralysis in 26.16 and 25.83min respectively and time of death of 45.83 and 45.33 min respectively. The Piperazine citrate exhibited the same at 19.33 and 22.6 min. respectively.These promising results in relation with pesticidal activity open the way for complementary investigation in order to purify and identify active molecules present in the leaf extract of *G. aborea*.

4 CONCLUSION

On the basis of the results obtained in the present study, it is concluded that *G. arborea* leaf extracts using different solvents (water, ethanol, and methanol) revealed the presence of different active compounds such as fats and oils, hydrolysable tannins, saponins, 2-deoxysugars, and free fatty acids. This implies that the plant can be a good source of treatment for diarrhea, extensive burns, bedsore, weeping ulcers and many other diseases that can be treated by these present bioactive compounds. The extracts of *G. arborea* were found to be effective antibacterial agents against human pathogens**.** This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Moreover, the *G. arborea* extracts showed a remarkable success in pesticidal screening thus, making this plant a potential substitute as an organic pesticide.

5 RECOMMENDATIONS

The pesticidal activity of alcoholic and aqueous extracts suggested that it is effective against sawfly (*Athalia lugens proxima)*. Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the pesticidal activity and study its pharmacological actions. It is also recommended that further studies on the plant alongside clinical trials to determine the potency of the plant as antimicrobial agents. The use of other parts of the *G. arborea* is also encouraged to further elucidate the presence of different bioactive compounds present in the plant. Furthermore, difference in the abundance of phytochemical composition due to geographical location, soil composition and age of the plant must be further determined.

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