Asian Journal of Biological and Life Sciences | Original Research

Antimicrobial Activities and Toxicities of the Leaf Extracts of *Ficus nota* (Blanco) Merr.

Felmer S. Latayada[1], Mylene M. Uy[2]*

1Department of Natural Sciences, Caraga State University, Butuan City, Philippines
2Department of Chemistry, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines

E-mail : mylene603@yahoo.com Contact No. : +63-63-2215041 loc 4123

Submitted : 25.07.2016 Accepted : 20.09.2016 Published : 30.12.2016

Abstract

The antimicrobial activities and cytotoxic potentials of the ethanol, hexane, chloroform and aqueous extracts of the Philippine medicinal plant *F. nota* was evaluated using disc diffusion assay and brine shrimp lethality assay, respectively. The aqueous extract (FnA) showed active to very active levels of inhibition against the three bacterial test organisms *B. subtilis, S. aureus,* and *E. coli*, with zones of inhibitions ranging from 18.7±1.15 mm to 20.0±0.00 mm. The extract FnA further exhibited activity against the two fungi *S. cerevisiae* (11.7±1.15 mm, partially active) and *C. albicans* (14.0±1.00 mm, active). This significant antimicrobial results of the aqueous extract was followed by the chloroform extract, FnC (17.0±5.2 mm, active against *B. subtilis* and 22.3±4.62 mm, very active against *S. aureus*). Meanwhile, the toxicity test showed that the ethanol extract FnE scored significant toxicities against the test organism *Artemia salina* with LC_{50} values of 79.43 g/ml and 206.5 g/ml, respectively. This study shows that the bioactive components of *F. nota* with antibacterial, antifungal, and potential cytotoxic properties were successfully and effectively extracted and concentrated in the various leaf extracts. These findings further support the ethno-medicinal uses of *F. nota.*

Key words : medicinal plant, cytotoxicity, antimicrobial, anticancer, synergistic effects

INTRODUCTION

Infectious diseases[1,2] and cancer[3,4] are the leading causes of morbidity and mortality around the world and continue to present major public health concerns. Today, many infections are caused by multi-resistant microorganisms that have resulted to a very demanding work to treat the diseases, and consequently increase the healthcare cost[1]. Likewise, the limited efficiency and the high cost of existing cancer therapies such as radiation, chemotherapy, and surgery are indications of the high mortality rate among cancer patients[4]. Nature provides a vast source of natural products[1] that has been historically used for treatment of various diseases, and researchers around the world have capitalized and made significant progress in the discovery of new antimicrobial and anticancer compounds[4]. Evidence of the success in natural product drug discovery is clear as more than 80% of drug substances are natural products or inspired by a natural product compound and over 100 natural-product-derived compounds are currently undergoing clinical trials with at least 100 similar projects in preclinical development in all major therapeutic areas[2,5]. However, despite of the successes, the anti-infective field is experiencing a shortage of lead compounds progressing into clinical trials[2] to control the spread of drug-resistant pathogens[6]. Meanwhile, in cancer chemotherapy, many cancer patients develop resistance to treatment with standard anti-cancer agents and this has become a serious problem[7]. Therefore, there is a pressing need for screening, isolation, and identification of new antimicrobial and anticancer agents from new sources including plants.

The Philippines has remarkable biodiversity and is rich in traditions of plant use. However, scientific studies and pharmacological investigations of Philippine medicinal plants just gained momentum recently[8]. The importance of the country's diverse medicinal plants lies not only in their chemotherapeutic value in traditional healthcare but also on the great prospect of finding a new source of bioactive chemical compounds[8]. *Ficus nota* is among the plants considered in this study. *F. nota* (Blanco) Merr., a member of family Moracea, is a small tree endemic to the Philippines and locally known as "tibig". The young leaves are used as vegetables when cooked and the ripe fruit can be eaten raw. It is used to treat fever by extracting the water from a standing tree and drunken daily and applied to ease muscle pain. The decoction of roots and bark and the water from cut branches are used to treat urinary tract infection, hypertension and diabetes[9]. A chemical investigation on the dichloromethane extract of the unripe fruits of *F. nota* afforded the following chemical constituents: 4-(2-hydroxyethyl)-2-methoxyphenol, a mixture of meso-2,3-butanediol, (2R,3R)-2,3-butanediol and (2S,3S)-2,3-butanediol and β-sitosterol[9]. Cytotoxicity study using *in vivo* brined shrimp lethality of the decoction and ethanol extracts of the stem of *F. nota* were active against the brine shrimp *Artemia salina* with LC_{50} values of 991.00 ppm and 852.22 ppm, respectively[10]. The decoction and ethanol extracts of the leaves of *F. nota* has been recently reported to possess considerable antioxidant properties[11]. However, there are no reports yet on the antimicrobial activities and potential cytotoxicities of the leaf extracts of *F. nota.* This study was, therefore, carried out to investigate the antimicrobial and cytotoxic efficacy of the leaf extracts of *F. nota* not only to establish the scientific basis for its traditional utilization as herbal medicine but also with the hope of providing vital information on the possibility of isolating bioactive chemical compounds that can be used as drug or drug leads. Additionally, these plants extracts could possibly be used to produce alternative forms of natural products that could act in synergism with conventional drugs to combat the growing threat of multi-drug resistant pathogens in anti-infective and anti-cancer therapies.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh and healthy leaf samples of the *F. nota* were collected within the surrounding area of Caraga State University,

Ampayon, Butuan City, Philippines. Identification and authentication of the collected plant material was done by Prof. Meljan T. Demetillo of the Dept. of Biology, College of Arts and Sciences, Caraga State University, Philippines. Voucher specimen is deposited at the laboratory of one of the authors. The fresh plant leaves were washed thoroughly under running water, air-dried for about two weeks, homogenized and stored in airtight containers until use. Appropriate amount of homogenized powdered plant materials were soaked in an adequate amount of 95% ethanol for 72 hours. The resulting mixture was filtered, concentrated *in vacuo* using rotary evaporator at temperature not exceeding 40^0 C, and weighed to provide the ethanol extract. A portion of the crude ethanol extract was then sequentially partitioned in hexane:water and chloroform:water solutions. The hexane-soluble, chloroform-soluble and water-soluble portions were then individually concentrated under *vacuo* and then weighed to get the extracts of hexane, chloroform and water, respectively. The plant extracts were coded as follows: FnE (*F. nota* ethanol extract), FnC (*F. nota* chloroform extract), FnH (*F. nota* hexane extract), and FnA (*F. nota* aqueous extract).

Microorganisms and culture media

The microorganisms were obtained from the Department of Science and Technology of the Philippine Government. The test microorganisms were as follows: (a) bacteria: *Staphylococcus aureus* No. 4, *Bacillus subtilis* No. 122, *Pseudomonas aeruginosa* No. 5, *Escherichia coli* No. 78, and (b) fungi: *Aspergillus flavus* No. 3006, *Aspergillus niger* No. 5540, *Candida albicans* No. 2049, and *Saccharomyces cerevisiae* No. 2006. Bacterial cultures were maintained on nutrient agar (NA) and *fungi* were maintained on Sabouraud dextrose agar (SDA).

Antimicrobial activity: Disc agar diffusion method

In vitro antimicrobial assay of the ethanol, hexane, chloroform, and aqueous extracts of *F. nota* was done using disc agar diffusion method[12] with minor modifications. Microbial suspensions of specific test microorganisms were swabbed separately onto plates containing nutrient agar (NA) for bacteria and Sabouraud dextrose agar (SDA) for fungi. Five minutes after swabbing, sterile paper disc with 6 mm in diameter, previously soaked separately in 10, 000- ppm concentration extracts, negative control (solvents: ethanol, hexane, chloroform, and water), and positive control (bacteria: Himox (5 mg/ml) for *B. subtilis* and *S. aureus*, Maxitrol (1mg/3.5mg/6000IU) for *E.coli* and *P. aeruginosa, f*ungi: Sporanox (10 mg/ml) for *A. flavus* and *A. niger*, Nystatin (100,000IU/ml) for *C. albicans* and *S. cerevisiae*) were placed in each agar test plate equidistant to each other by carefully dropping them in with a sterile pointed dental probe, and then pressing them on the agar very lightly. The paper disc containing the test fractions and extraction solvents were allowed to dry for a few minutes before introducing into the agar plates. The seeded agar plates with impregnated paper discs were stored for about 6 h in the refrigerator to allow diffusion of the impregnated material onto the agar. The plates were then incubated, upside down, 24 h (for plates seeded with bacteria) and 48 h (for plates seeded with fungi) at 37^0 C after which the zones of inhibition were then measured. The measurement was done by taking the total diameter of the zone (including the disc within) in mm, at 2 perpendicular diameters then the average was calculated. Three trials with two replicates were done on each of the *F. nota* extracts.

Toxicity assay: Brine shrimp lethality test

Brine shrimp lethality bioassay[13,14] was carried out to investigate the potential cytotoxicity of the leaf extracts of *F. nota.* The eggs of brine shrimp (*A. salina* Leach) was introduced on the other side of the small compartmentalized tank with a black-tinted glass divider filled with filtered and sterile sea water, covered with clean aluminum foil and fully aerated at room temperature. Drops of yeasts solution were added to both sides of the compartments to supply food for the resulting nauplii. The other side of the compartment was left open to allow contact with air and illuminated. After 48 hours, the newly-hatched nauplii were attracted and moved to the illuminated compartment and were collected with a Pasteur pipette. Briefly, stocks solutions (10 mg/ml) of all the plant fractions were prepared by dissolving them in their respective solvents. Different levels of concentrations (1000, 500, and 100 g/ml) were prepared by drawing different volumes from the stock solutions and added into the test tubes, dried using high purity nitrogen gas, dissolved in dimethyl sulfoxide (DMSO) (0.6%) and then added with 4 ml filtered and sterilized sea water. Ten brine shrimps nauplii were added to each of the test tube. The volume was then adjusted to 5 ml with filtered and sterilized sea water. Each level of concentration was tested in triplicates. The negative control contained brine shrimp, filtered and sterilized sea water and DMSO (0.6%) only. All the prepared test tubes were maintained under illumination and number of dead and alive larvae was counted after 24 hours. The toxicity results of the plant extracts were evaluated by calculating the percent mortality of the brine shrimps using the equation,

% Mortality= (Total no. of dead brine shrimps) / (Total no. of brine shrimps) x 100

and the LC_{50} values were then determined using the Reed-Muench method.

RESULTS

Antimicrobial activity

The antimicrobial activity of the leaf extracts of *F. nota* against different representative test organisms (bacteria and fungi) was evaluated and expressed in terms of zones of inhibition in mm. The results of this assay are presented in Tables 1 and 2.

Toxicity assay

In this assay, the cytotoxicity of the leaf extracts of F. nota were measured against the brine shrimp *A. salina* and expressed as percent mortality and LC_{50} values. Table 3 summarizes the results.

DISCUSSION

Antimicrobial activity

The *F. nota* leaf extracts showed varying levels of antibacterial activities. The ethanol extract of *F. nota* (FnE) showed very active of inhibition only against *S. aureus*. Surprisingly, the aqueous extract FnA had a remarkable range of activity against the four test bacterial organisms. FnA was able to inhibit the three bacterial organisms *E. coli, B. subtilis,* and *S. aureus* with zones of inhibitions ranging from 18.7±1.15 mm to 20.0±0.00 mm that fall under active to very active levels of inhibitory activity. Moreover, the plant's chloroform extract, FnC was active against *B. subtilis* and very active against *S. aureus.*

Table 1: Antibacterial activity of *F. nota* leaf extracts and positive controls.

Test samples	Zone of inhibition*, mm*****			
	E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*
FnE	(–)	(–)	20.7±2.08	(–)
FnH	(–)	(–)	(–)	(–)
FnC	9.0±0.00	17.0±5.2	22.3±4.62	8.3±0.58
FnA	18.7±1.15	20.0±0.00	19.7±1.53	(?)
Himox**	Nd	43.3±2.8	43.3±5.77	Nd
Maxitrol***	39.3±0.58	Nd	Nd	18.5±1.32
Ethanol****	(–)	(–)	(–)	(–)
Chloroform****	(–)	(–)	(–)	(–)
Hexane****	(–)	(–)	(–)	(–)
Water****	(–)	(–)	(–)	(–)

* - mean of 3 replicates and expressed as mean ± SD
** - positive control for Gram (+) bacteria
*** - positive control for Gram (-) bacteria
**** - Negative control
(–) - No activity
Nd - not determined
***** - <10 mm: inactive; 10 mm 13 mm: partially active; 14 mm 19 mm: active; >19 mm: very active

Table 2: Antifungal activities of *F. nota* leaf extracts and positive controls.

Test samples	Zone of inhibition*, mm*****			
	A. niger	*A. flavus*	*C. albicans*	*S. cerevisea*
FnE	(–)	(–)	(–)	(–)
FnH	(–)	(–)	(–)	(–)
FnC	(–)	(–)	(–)	(–)
FnA	10.0±0.00	9.0±3.46	14.0±1.00	11.7±1.15
Sporanox**	17.0±2.6	25.0±1.0	nd	nd
Nystatin***	nd	nd	19.2±1.44	20.0±0.00
Ethanol****	(–)	(–)	(–)	(–)
Chloroform****	(–)	(–)	(–)	(–)
Hexane****	(–)	(–)	(–)	(–)
Water****	(–)	(–)	(–)	(–)

* - mean of 3 replicates and expressed as mean ± SD
** - positive control for fungi
*** - positive control for fungi
**** - Negative control
(–) - No activity
Nd - not determined
***** - <10 mm: inactive; 10 mm 13 mm: partially active; 14 mm 19 mm: active; >19 mm: very active

However, the plant hexane extract FnH exhibited no inhibition or activity against all the test organisms. Meanwhile, *P. aeruginosa* was not inhibited by any of the plant extracts. The negative controls showed no effect against the test bacterial organisms which suggest the efficacy of the plant extracts. The ethanol extract of the plant, FnE, has no activity against all the fungi test

Table 3: Effects of F. nota leaf extracts on *A. salina* after 24 hour-exposure.

Plant Extract	Concentration of Plant Extract	Brine shrimp Mortality (%)	Chronic LC_{50} (μg/ml)
FnE	100	57.14	79.43
	500	90.0	
	1000	100.0	
FnH	100	11.6	333.0
	500	67.5	
	1000	91.2	
FnC	100	28.9	206.5
	500	80.48	
	1000	100.0	
FnA	100	0.0	588.8
	500	42.5	
	1000	78.72	
	500	0.0	
	1000	0.0	

organisms. However, the aqueous extract FnW showed inhibitions against two fungi. FnA was partially active against *S. cerevisea* and active against *C. albicans*. FnA was inactive against *A. niger* and *A. flavus*. The negative controls showed no effect against the fungi test organisms which suggest the efficacy of the aqueous extract of *F. nota* (FnA).

If active constituents are present in high amounts, it is possible that other constituents exert antagonistic effects on the bioactive compounds[15]. It was observed that, to some extent, the hexane extracted components (FnH) may have contributed antagonistic effects[16] to the ethanol extract FnE on its antimicrobial activity which resulted to the selective activity of FnE among the test organisms. The combined activity of all the components in the ethanol extract of the plant was less than those expected for the extracts partitioned from it. On the other hand, synergistic effects[16] of the individual chemical components might be the driving force that made FnA and FnC more effective. These plant extracts (FnA and FnC) are still in crude state and they may contain two or more bioactive chemical components. These results indicate that the antimicrobial components of both plant samples were effectively extracted and concentrated in the aqueous and chloroform solvents and have the potential for further investigation.

Toxicity assay

The potential cytotoxic property of the *F. nota* leaf extracts was evaluated using the brine shrimp (*A. salina*) lethality bioassay (BSLT). The method is simple, rapid, reliable, inexpensive, and convenient[13]. The method has been applied to plant extracts in order to facilitate the isolation of biologically active components[17]. Since its initiation in 1982, with minor modification in 1991[18], it has been successively employed for bioassay-guided fractionation of active cytotoxic and antitumor agents such as trilobacin from *Asimina triloba*, *cis*-annonacin from *Annona muricata,* and *ent*-kaur-15-en-19-oic acid from *Elaeoselinum foetidum*[17]. In this study, all tested plant extracts displayed toxicity (LC_{50} <1000 g/ml)[13] against the brine shrimp test. The crude ethanol extract FnE and the chloroform extract FnC both scored LC_{50} values of less than 250 g/ml which are considered significantly active[17,18] and warrants further investigation. Furthermore, the potency exhibited by these two extracts are even higher than those of the decoction and ethanol extracts of the plant stem[10].

CONCLUSION

This study has shown that the aqueous and chloroform extracts of the leaves of *F. nota* possess antibacterial, antifungal, and cytotoxic properties. These findings suggest that *F. nota* is a potential source of bioactive natural compounds.

ACKNOWLEDGMENTS

The authors acknowledge the Commission on Higher Education Faculty Development Program II (CHED-FDP II) and the Department of Science and Technology-Philippine Council for Health Research and Development (DOST-PCHRD) of the Philippine government for the support.

REFERENCES

1. Hayashi MA, Bizerra FC, Da Silva Jr PI. Antimicrobial compounds from natural sources. Front. Microbiol. 2013:4:195.

2. Lam KS. New aspects of natural products in drug discovery. Trends in Microbiology. 2007:15(6):279-289.

3. Park SU. Anticancer Compounds from Plants. EXCLI Journal. 2012:11:386-389.

4. Talib WHadi. Anticancer and Antimicrobial Potential of Plant-Derived Natural Products, Phytochemicals - Bioactivities and Impact on Health, Prof. Iraj Rasooli (Ed.), DOI: 10.5772/26077. InTech Europe; Croatia, 2011.p141-158.

5. Harvey AL. Natural products in drug discovery. Drug Discovery Today. 2008:13(19-20):894-901.

6. Srivastava J, Chandra H, Nautiyal AR, Kalra SJS Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. Biotechnology. 2014:4:451460.

7. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. Journal of Ethnopharmacology. 2005:100:7279.

8. Vital PG, Rivera WL. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts. Journal of Medicinal Plants Research. 2009:3(7):511-518.

9. Ragasa CY, Alimboyoguen AB, Shen CC. Chemical Constituents of *Ficus nota*. *Der* Pharma Chemica, 2014:6(4):98-101.

10. Arquion CD, Nuñeza OM, Uy MM. Evaluating the Potential Cytotoxic Activity of *Ficus nota* Using Brine Shrimp Lethality Test. Bulletin of Environment, Pharmacology and Life Sciences, 2015:4(12):40-44.

11. Latayada FS, Uy MM. Screening of the Antioxidant Properties of the Leaf Extracts of Philippine Medicinal Plants *Ficus nota* (Blanco) Merr., *Metroxylon sagu* Rottb., *Mussaend philippica* a. Rich., *Inocarpus fagifer*, and *Cinnamomum mercadoi* Vidal. Bulletin of Environment, Pharmacology and Life Sciences, 2016:5(3):18-24.

12. Guevara BQ. A Guidebook to Plant Screening: Phytochemical and Biochemical Revised Ed. Research Center for the Natural Sciences, University of Santo Tomas: Manila, Philippines: Research Center for the Natural Sciences, University of Santo Tomas; Manila Philippines, 2005. p. 71-76.

13. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Journal of Medicinal Plant Research, 1982:45:31-34.

14. Peteros NP, Uy MM. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Journal of Medicinal Plant Research, 2010:4(5):407-414.

15. Kowti R, Harsha R, Ahmed MG, Hareesh AR, Thammanna Gowda SS, Dinesha R, Satish Kumar BP, Irfan Ali M. Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata* P. Beauv. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010:1(3):691- 698.

16. Nelson AC, Kursar TA. Interactions among plant defense compounds: a method for analysis. *Chemoecology*, 1999:9:81-92.

17. Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O. Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. Naresuan University Journal, 2004:12(2):13-18.

18. Reiser MJ, Gu Z-M, Fang X-P, Zeng L, Wood KV, McLaughlin JL. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. Journal of Natural Products, 1996:59:100-108.

19. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology, 2001:74:113123.

20. El-Zawahry YA, Reda FM, Azazy WM. Synergistic Effect of Combination Treatment by Certain Plant Extracts and Some Antibiotics on the Resistance of Pathogenic Bacteria to Some Common Antibiotics. Life Science Journal, 2013:10(4):3477-3489.