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Original article

ASSESSMENT OF ANTIOXIDANT, ANTIMICROBIAL AND CYTOTOXIC PROPERTIES OF FRUITS OF *Melocanna baccifera* (ROXB.) KURZ

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Abstract

The crude methanolic extract of the fruits of Melocanna baccifera (Roxb.) Kurz and its different organic soluble Kupchan fractions were screened for antioxidant, antimicrobial and cytotoxic properties. The antioxidant effect was measured by 1,1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay and by the determination of total phenolic content (TPC). The polyphenol content was assessed colorimetrically using Folin-Ciocalteau method and expressed as gallic acid equivalent. Evaluation of antimicrobial and cytotoxic property was performed by using the disc diffusion method and brine shrimp lethality bioassay, respectively. The highest total phenolic was found in the aqueous soluble fraction (142.8 mg of GAE/g of dried extract) demonstrating the significant antioxidant potentials. In DPPH assay for free radical scavenging property, the aqueous soluble fraction of fruit of M. baccifera exhibited significant antioxidant activity with the IC_{50} value of 28.5 µg/mL as compared to 23.50 µg/mL revealed by standard butylated hydroxyl toluene. In brine shrimp lethality bioassay, the crude methanol extract exhibited moderate cytotoxic activity having LC_{50} value 34.47 µg/mL as compared to vincristine sulphate (LC_{50} 5.31 µg/mL). In antimicrobial screening, the average zone of inhibition produced by the crude methanol extract and the dichloromethane soluble fraction were found to be 7.0-12.0 and 7.4-12.2 mm, respectively at a concentration of 400 µg/disc. A positive correlation was observed between total phenolic content and free radical scavenging activity having correlation coefficient (R^2)of 0.80. These results suggested that M. baccifera could be considered as a source of bioactive secondary metabolites having antioxidant properties and effective protection from free radicals.

Key words: *Melocanna baccifera, Antioxidant, Total phenolic content, Free radical scavenging, Antimicrobial activity, Cytotoxic activity, Brine shrimp lethality bioassay.*

Melocanna baccifera (Roxb.) Kurz Meyvelerinin Antioksidan, Antimikrobiyal ve Sitotoksik Özelliklerinin Değerlendirilmesi

Melocanna baccifera (Roxb.) Kurz meyvelerinin metanollü ham ekstresi ve ekstrenin farklı organik çözünür Kupchan fraksiyonları antioksidan, antimikrobiyal ve sitotoksik özellikleri bakımından taranmıştır. Antioksidan etki 1,1-difenil-2-pikrilhidrazil-hidrat (DPPH) serbest radikal süpürücü test (TPC) ve total fenolik içeriğin tayini ile ölçülmüştür. Polifenol içeriği Folin-Ciocalteau metodu kullanılarak kolorimetrik olarak belirlenmiş ve gallik asite eşdeğer olarak ifade edilmiştir. Antimikrobiyal ve sitotoksik özelliklerin değerlendirilmesi sırasıyla disk diffüzyon metodu ve tuzlu su karidesi letalite testleri kullanılarak ölçülmüştür. Belirgin antioksidan potensiyali gösteren total fenol içeriği en yüksek suda çözünebilen fraksiyonda (142.8 mg of GAE/g kuru ekstre)bulunmuştur. Serbest radikal süpürücü etkinin test edildiği DPPH testinde, M. baccifera meyvesinin suda çözünebilen

fraksiyonu 23.50 µg/mL IC_{50} değeri ile standart olarak kullanılan butil hidroksil toluen için ölçülen 28.5 µg/mL ile kıyaslandığında, belirgin antioksidan aktivite göstermiştir. Tuzlu su karidesi letalite testinde, ham metanol ekstresi 34.47 µg/mL LC_{50} değeri ile vinkristin sülfat ile kıyaslandığında (LC_{50} 5.31 µg/mL) orta sitotoksik aktiviteye sahip bulunmuştur. Antimikrobiyal taramada, 400 µg/disk konsantrasyonda ham metanol ekstresi ve diklorometan'da çözünebilen fraksiyon için ölçülen ortalama inhibisyon zonu sırasıyla 7.0-12.0 ve 7.4-12.2 mm olarak bulunmuştur. Total fenol içeriği ve serbest radikal süpürücü aktivite arasında 0.8 korelasyon katsayısına (R^2) sahip pozitif bir korelasyon gözlenmiştir. Bu sonuçlar M. baccifera'nın antioksidan özellikler ve serbest radikallerden koruyucu etkiye sahip biyoaktif sekonder metabolitlerin kaynağı olarak düşünülebileceğini göstermiştir.

Anahtar kelimeler: *Melocanna baccifera, Antioksidan, Total fenol içeriği, Serbest radikal süpürücü, Antimikrobiyal aktivite, Sitotoksik aktivite, Tuzlu su karidesi letalite testi.*

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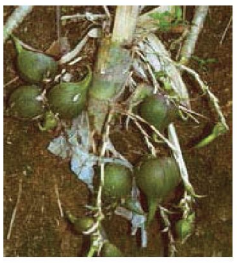
INTRODUCTION

Plants are known to produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of bioactive compounds, have continued to play dominant role in the maintenance of human health since ancient times (1). Over 50% of all modern drugs used clinically are originated from natural products (2) and they play an important role in drug development programs in the pharmaceutical industries (3).

Melocanna baccifera (Roxb.) Kurz (Bengali name: muli bash, Family: Graminae) is a thin-walled, small diameter and non-clump forming bamboo. It naturally grows throughout Bangladesh (4). The plant is known to flower gregariously at intervals of 40-44 years (5).



Clusters of developing muli fruits

Seed kernel

The seeds of this plant have been reported to increase the fertility in rats or enhance human libido (6). Previous phytochemical investigation of the fruit of this plant led to the isolation of aromatic compounds *i.e.* isochavicinic acid, eugenol; pentacyclic triterpene *i.e.* olean-12-en-28-carboxy-3β-acetate, 3-oxo-olean-12-en-28-al and glycoside compounds *i.e.* β-sitosterol glucoside and stigmasterol glucoside for the first time (7). In the present study, the organic soluble materials of the methanol extract of the fruit of M. *baccifera* and its different organic soluble partitionates were evaluated for their antioxidant, antimicrobial and cytotoxic properties.

MATERIALS AND METHODS

Plant materials

The fruits of *Melocanna baccifera* (Roxb.) Kurz were collected from Dhaka in June, 2008 and properly identified at Department of Botany, University of Dhaka and Bangladesh National Herbarium, where a voucher specimen (Accession no: DACB-36157) has been deposited for future reference. The fruits were cut into small pieces and shade dried for 7 days followed by oven drying for 24 hours at 40°C to facilitate proper grinding.

Extraction and isolation

The powdered material (533 g) was soaked in 1.5 liter of methanol for 7 days with occasional shaking and stirring. The methanol phase was then filtered off through a cotton plug followed by Whatman filter paper no.1 and the filtrate thus obtained was concentrated at 40°C using a rotary evaporator (Brand name-Heidolph, Germany). A portion (5.0 g) of the crude methanol extract was fractionated by the modified Kupchan partitioning protocol (8) which afforded the *n*-hexane (650 mg), carbon tetrachloride (750 mg), dichloromethane (350 mg), and aqueous (3.05 g) soluble materials.

Antioxidant Activity

Total phenol content (TPC) analysis

Total phenolic content of the extracts of *M. baccifera* was measured by employing the method (9) involving Folin-Ciocalteau reagent as an oxidizing agent and gallic acid as a reference. 2.5 mL of Folin-Ciocalteau reagent (diluted 10 times with water) and 2.0 mL of sodium carbonate (7.5% w/v) solution were added to 0.5 mL of extract solution (2 mg/mL) in methanol. After 20 minutes of incubation at room temperature, the absorbance was measured at 760 nm using a UV-visible spectrophotometer (Brand name-Shimadzu, Japan). Total phenolics were quantified by calibration curve obtained from measuring the known concentrations of gallic acid (0-100 µg/mL) and were expressed as mg of GAE (gallic acid equivalent)/g of the dried extract.

Free radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams *et al.* (10). 2.0 mL of a methanol solution of the sample (extract/standard) at different concentrations (500 µg/mL to 0.977 µg/mL) were mixed with 3.0 mL of a DPPH (20 µg/mL) in methanol. After 30 min of reaction at room temperature in dark place, absorbance was measured at 517 nm against methanol as blank by a UV-Visible spectrophotometer (Brand name-Shimadzu, Japan). Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material) and A_{sample} is the absorbance of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against extractive/standard concentration.

Antimicrobial assay

The antimicrobial activity of the Kupchan fractions was determined by the disc diffusion method (11) against a number of Gram positive and Gram negative bacteria and fungi. The bacterial and fungal strains used in this experiment were collected as pure cultures from the

Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The samples were dissolved separately in chloroform and applied to sterile discs at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent. Standard kanamycin disc (30 µg/disc) was used as the positive control in the experiment.

Cytotoxic activity

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (12). The simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. For cytotoxicity screening, DMSO (dimethyl sulfoxide) solutions of the extract were applied to *Artemia salina* in one day *in vivo* assay. For the experiment, 4 mg of each extract was dissolved in DMSO and the solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.123, 1.563, 0.781 µg/mL) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live brine shrimp nauplii in 5 mL simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the samples was obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration.

Statistical analysis

For each extract, three samples were prepared for each of the bioassay. The total phenolic content, IC_{50}, LC_{50} values and the zone of inhibition were calculated as mean ± SD (n=3) for the total phenol content, antioxidant, cytotoxic activity and antimicrobial screening, respectively.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the antioxidant, antimicrobial and cytotoxic activities of various extracts of the fruits of *M. baccifera* and the results are summarized in Tables 1 and 2, and Figure 1. The total phenolic content varied for different Kupchan fractions of the methanolic extract ranging from 40.05 mg to 142.8 mg of GAE/g of the dried extract (Table 1). The highest total phenol content was found in the aqueous soluble fraction (142.8 mg of GAE/g of dried extract) demonstrating the significant antioxidant potentials. The dichloromethane soluble Kupchan fraction and the crude methanolic extract revealed moderate antioxidant activity.

The correlation analysis revealed that a correlation exists between total phenolic content and free radical scavenging activity. The correlation coefficient (R^2) for the total phenolic content and free radical scavenging activity (Figure 1) was 0.75 indicating a positive relationship. This result suggests that 75% of the free radical scavenging activity resulted from the contribution of the phenolic compounds (13). Different secondary metabolites may also contribute to the antioxidant capacity, which in this case contributed to approximately 20% of the antioxidant activity (14).

Table 1. Total phenolic content, free radical scavenging, cytotoxic activities of test samples of *M. baccifera.*

Sample	Total Phenolic Content (mg of GAE/g of dried extract)	IC_{50} (µg/mL)	LC_{50} (µg/mL)
BHT	Not determined	23.50 ± 0.32	Not determined
VS	Not determined	Not determined	5.31 ± 1.25
CM	102.7 ± 0.75	55.0 ± 1.29	34.47 ± 1.20
HSF	40.05 ± 0.22	295.50 ± 0.96	64.57 ± 0.95
CTSF	77.12 ± 1.35	70.00 ± 1.33	85.57 ± 1.33
DMSF	110.5 ± 0.78	48.50 ± 1.25	52.10 ± 0.85
AQSF	142.8 ± 1.25	28.0 ± 1.14	67.99 ± 1.15

The average values of three calculations are expressed as mean ± SD (Standard deviation); VS = Vincristine sulphate; BHT = Butylated hydroxy toluene; CM = Crude methanolic extract; HSF = *n*-hexane soluble fraction; CTSF = Carbon tetrachloride soluble fraction; DMSF= Dichloromethane soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract of *M. baccifera.*

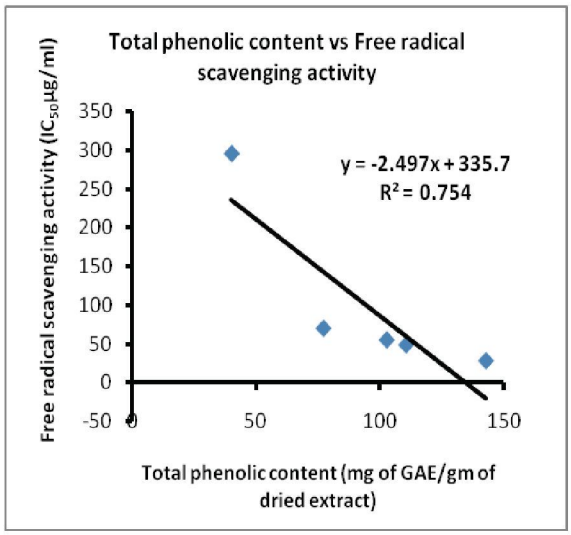


Figure 1. Correlation between the total phenolic content and free radical scavenging activity

In the present study, only the crude methanol extract and dichloromethane soluble fraction showed mild to moderate antimicrobial activity against most of the pathogens examined. The average zones of inhibition produced by the methanolic crude extract and the dichloromethane soluble fraction were found to be 7.0-12.0 and 7.4-12.2 mm respectively, at a concentration of 400 µg/disc (Table 2). The crude extract showed significant activity against *Shigella boydii* while the dichloromethane soluble fraction was found to be most active against *Bacillus megaterium*. The hexane and carbon tetrachloride soluble fraction showed no activity against any of the test organisms (data not shown).

Table 2. Antimicrobial activity of test samples of *M. baccifera.*

Test Bacteria and Fungi	Diameter of Zone of Inhibition (mm)		
	CM	DMSF	Kanamycin (30 µg)
Gram-Positive			
Bacillus cereus	8.0 ± 0.46	9.0 ± 0.32	32.4 ± 0.50
B. megaterium	7.8± 0.15	12.2 ± 0.25	33.3 ± 1.10
B. subtilis	8.2± 0.49	9.5 ± 0.60	30.0 ± 1.00
Staphylococcus aureus	10.5± 0.25	8.5 ± 0.25	29.6 ± 0.49
Sarcina lutea	10.5± 0.20	10.4 ± 0.35	33.7 ± 1.20
Gram-Negative			
Escherichia coli	7.5± 0.66	11.2 ± 0.42	32.0 ± 0.49
Pseudomonus aeruginosa	8.4± 0.21	8.5 ± 0.25	32.4 ± 0.40
Salmonella typhi	7.0± 0.44	10.9 ± 0.51	32.0 ± 1.00
S. paratyphi	11.0± 0.15	8.4 ± 0.49	30.8 ± 0.90
Shigella boydii	12.0± 0.30	7.4 ± 0.15	33.0 ± 1.00
S. dysenteriae	10.4± 0.20	10.7 ± 0.10	35.5 ± 0.50
Vibrio mimicus	8.5± 0.51	8.4 ± 0.46	30.7 ± 0.40
V. parahemolyticus	7.0± 0.40	9.6 ± 0.15	32.3 ± 0.30
Fungi			
Candida albicans	9.2± 0.15	8.1 ± 0.32	36.0 ± 1.00
Aspergillus niger	8.0± 0.25	10.7 ± 0.10	36.7 ± 1.50
Saccharomyces cerevisiae	8.0± 0.66	8.6 ± 0.26	35.5 ± 0.50

In cytotoxicity test, % mortality increased gradually with the increase in concentration of the test samples. LC_{50} values obtained from the best-fit line slope were 5.31, 34.47, 64.57, 85.57, 52.10 and 67.99 µg/mL for standard vincristine, crude methanol extract, *n*-hexane, carbon tetrachloride, dichloromethane and aqueous soluble fractions, respectively, (Table 1). In comparison to positive control (vincristine sulphate), the crude methanol extract exhibited moderate cytotoxic activity while the *n*-hexane, carbon tetrachloride, dichloromethane and aqueous soluble fractions demonstrated mild cytotoxic activity. The cytotoxicity exhibited by the extract of *M. baccifera* suggests the presence of antimicrobial, pesticidal, antitumor, anthelmintic entities.

CONCLUSION

From the above results, it can be concluded that the different extracts of *Melocanna baccifera* possess significant DPPH radical scavenging activity and mild to moderate antimicrobial activity which also suggest the presence of bioactive secondary metabolites. Previously we have reported the isolation of aromatic compounds, pentacyclic triterpene and glycosides for the first time from this plant (7). However they could not be tested because of the scarcity of the samples. Therefore, the plant could be subjected to extensive bio-assay guided chromatographic separation and purification processes for isolation of active molecules for the discovery of novel therapeutic agents.

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