American Journal of Bio Science
2015; 3(2-1): 19-22
Published online February 26, 2015 (http://www.sciencepublishinggroup.com/j/ajbio)
doi: 10.11648/j.ajbio.s.2015030201.14
ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online)

Potential In-Vivo Evaluation of Analgesic Investigation of *Mangifera indica* and Antimicrobial Activity of *Areca catechu*

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To cite this article:
Mst. Marium Begum, Taslima Begum, Mohammad Ashikur Rahman, Tahmina Haque, Md. Abir Khan, Md. Belal Hossain, Hasan Tarek, Md. Noor-A-Alam, Md. Reyad-ul- Ferdous, Md. Safkath Ibne Jami. Potential In-Vivo Evaluation of Analgesic Investigation of *Mangifera indica* and Antimicrobial Activity of *Areca catechu*. *American Journal of Bio Science*. Special Issue: Pharmacological and Phytochemicals Investigation. Vol. 3, No. 2-1, 2015, pp. 19-22. doi: 10.11648/j.ajbio.s.2015030201.14

Abstract: This project report describes the biological activity of the dried leaves of *Mangifera indica* belonging to the family Anacardiaceae and the dried fruits of *Areca catechu* belonging to the family Arecaceae. The dried powders of leaves and fruits were extracted with organic solvents –Carbon tetrachloride, Methanol & Pet ether sequentially by maceration process. After this, The crude extracts of Carbon tetrachloride, Methanol & Pet ether were investigated for analgesic and antimicrobial property. For *Mangifera indica*, the crude extracts of Carbon tetrachloride, Methanol & Pet ether were studied for analgesic property at an oral dose of 200 mg /kg of body weight using acetic acid induced writhing effect method. The result showed that the Methanol and Pet ether extracts had mild analgesic property (having a writhing inhibition of 51.7% and 50% respectively), while carbon tetrachloride extract did not show significant analgesic property (having a writhing inhibition of 30 %). For *Areca catechu*, the crude extracts of Carbon tetrachloride, Methanol & Pet ether were screened for antimicrobial activity against gram positive and gram negative bacteria and fungi using disk diffusion method. The results obtained were compared with that of standard drug kanamycin. The Carbon tetrachloride extract showed mild sensitivity to several gram positive, gram negative bacteria & Fungi (zone of inhibition 9-10 mm). The Methanol extract also showed mild sensitivity to several gram positive, gram negative bacteria & Fungi (zone of inhibition 7-10 mm).and slightly to highly sensitive to fungi (zone of inhibition 8-40 mm). The pet ether extract Crystal (Found after adding Carbon tetrachloride) showed mild sensitivity to only a gram negative bacteria (*Shigella boydii*) having zone of inhibition 7 mm.

Keywords: *Mangifera indica*, Anacardiaceae, *Areca catechu* Arecaceae, Antimicrobial Activity, Disc Diffusion Method, Analgesic Activity, Writhing Effect

1. Introduction

Inflammation is one common and major cause of sufferings now and every time past. Those drugs that are available are known as NSAID, i.e. non-steroidal anti-inflammatory drugs, act by inhibiting the function of prostaglandin. Prostaglandin is an autocoid that release extracellularly and initiate pain. Anti-inflammatory agents either block this autocoid synthesis by inhibiting COX enzyme or protecting lysosomal membrane from break down. Plant is a source of wide variety of chemicals. Most of them need to be synthesized. One plant may consist of several compounds that have several effects on physiology. The main source of medicine from the beginning of mankind till

modern time is plant[1]. In Ayurvedic and other traditional medicinal practices the plant has been used against diseases like bilious complaints, cough, worms, jaundice, fever, inflammation, rheumatism, anaemia and vermifuge. Phytochemicals like alkaloids, flavonoids, terpenes and sterols have been isolated [2]. Many people cannot afford drugs and rely on medicinal plants and traditional medicines for health care [3]. More than five hundred plants growing in Bangladesh are reputed to be medicinally effective in many disease conditions of health [4].Medicinal plants of Bangladesh have a great prospect for drug developments. This requires extensive research on the indigenous plants. The genus *Mangifera* consisting of 69 species [5] under the family of Anacardiaceae and the genus *Areca* consisting of 50 species under the family of Arecaceae possess several medicinal properties bearing scientific basis. *Mangifera indica* (Bengali name: Aam*)* and *Areca catechu* (Bengali name: Supari*),* grows in the tropical region of Asia including Bangladesh is used medicinally in various disease conditions [6]. The aim of the present work was to investigate the leaves of *Mangifera indica* and whole fruit part of *Areca catechu* for analgesic and antimicrobial properties respectively.

2. Methods and Materials

2.1. Sample

Fresh fruits of leaves of *Mangifera indica* and whole fruit part of *Areca catechu* were collected from Narayangonj on December 2008 and identified by Botany Department of Dhaka University. The leaves and fruits were sliced and sun dried. Finally the dried leaves and fruits were ground into coarse powders.

2.2. Extraction

About 60 gm of the powdered material of was taken in a clean container and soaked in 700 ml of Methanol. The container with its content was sealed by foil and kept for a period of 9 days accompanying occasional shaking and stirring. The whole mixture was then filtered, first through cotton and then though filters paper. The filtrate thus obtained was kept in room temperature in a beaker with protective measure from dust and the solvent is allowed to evaporate leaving the extractive along. Same extraction process was followed for both powdered materials. The total amount of crude Methanol extract was 5.844 gm (*Mangifera indica*) and 7.326 gm (*Areca catechu*)[7].

2.3. Solvent-Solvent Partitioning

The Solvent-Solvent partitioning was done using the protocol designed by Kupchan and modified by Wagnen et al. (1993). The crude extract (5.844 gm) was dissolved in 10% aqueous Methanol. It was extracted with Pet ether (100 ml x 2), and then with Carbon tetrachloride (100 ml x2) .Same procedure was carried out for both the plant extract[8].

2.4. Study of Analgesic Property

Analgesic property was studied for *Mangifera indica*. Swiss-albino mice of either sex aged 4-5 weeks, weight 25 grams, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDRB) were used for the experiment. The animals were kept in polyvinyl cages at room temperature under normal light and dark condition and fed ICDDRB formulated rodent food and water *ad libitum.* The food and water were withdrawn 12 hours before the experiment to keep the hydration rate constant.

Crude extracts of Methanol, Pet ether and carbon tetrachloride at dose of 200 mg/kg body weight of mice were separated measured and triturated with few drops of Tween-80. After proper mixing of extract with Tween-80, normal saline was added slowly. The final volume of the suspension was made 5ml. To stabilize the suspension, it was stirred well with a vortex mixture. For the preparation of standard drug sample, diclofenac at the dose of 40-mg/kg-body weight, 10 mg of diclofenac was taken and a suspension of 5 ml was made with required amount of Tween-80 and saline solution.

The analgesic properties of the crude extracts were studied using acetic acid induced writhing effect method [9]. At zero hour, test samples, control (1% Tween-80 solution in saline) and diclofenac were administered orally with a feeding needle. After 40 minutes acetic acid (0.7%) 0.1ml/10g body weight basis of mice was administered intra-peritoneally to each of the animals of all the groups. Five minutes after the administration of acetic acid, number of squirms or writhing were counted over each mouse for 10 minutes.

2.5. Study of the Antimicrobial Property

Antimicrobial property was studied for *Areca catechu*. The disc diffusion method [8,10] was used to test antimicrobial activity of the extractives against five gram-positive and eight gram-negative bacteria and three fungi (Table-2). Solutions of known concentration (200 g/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of the solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette and the residual solvents were completely evaporated. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. Those plates were then kept at low temperature (4^oC) for 24 hours to allow maximum diffusion. There was a gradual change of test materials concentration in the media surrounding the discs. The plates were then incubated at 37^oC for 24 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by

measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out in triplicate.

2.6. Statistical Analysis

The results were expressed as the mean ± standard deviation (SD). Statistical significance of the mean mortality at each concentration was analysed using one-way analysis of variance (ANOVA) and compared using Duncan's multiple range tests. Values of $p \leq 0.05$ were taken to be statistically significant.

3. Results and Discussion

The peripheral analgesic activity of ethanolic and the chloroform extracts at a dose of 200mg /kg were determined with the acetic acid induced writhing inhibition method [11]. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A *P* value <0.05 was considered significant. The results of the animal experiments are shown in table 1. The ethanol and the chloroform extracts of the fruits of *Mangifera indica* at a dose of 200 mg /kg showed significant antinociceptive activity with 44.5% and 41.6% inhibition of writhing response respectively and mild antinociceptive activity with carbon tetrachloride extract (25% inhibition of writhing response). The results were statistically significant (ethanol extract - P < 0.02, chloroform extract - P < 0.02, carbon tetrachloride extract –P<0.05) in comparison to the control. As the crude extracts appeared to be active acetic acid induced animal models of nociception, might contain peripherally active antinociceptive values.

Table 1. *Effects of crude extracts[a] on acetic acid induced writhing response in mice.*

Animal group	Dose (mg/kg, p.o.)	Writhing[b]	% inhibition
Control (group V)		10 ± 0.707	0
Standard (group IV)	40	2.5 ± 0.540*	75
Ethanol extract (group I)	200	5.55 ± 0.320**	44.5
Chlororform extract (group II)	200	5.83 ± 0.442**	41.6
Carbon tetrachloride extract (group III)	200	7.5 ± 0.353***	25

[a]1hr after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 5 minutes after the injection, the number writhing was counted for 15 min. [b]Values are mean ± SEM (n = 4); *$P<0.005$, **$P<0.02$, ***$P<0.05$ compared to control.

The crude extracts were tested for antimicrobial property by using disk diffusion method.

The results of antimicrobial tests were compared with the standard antimicrobial drug (kanamycin). The result of antimicrobial tests has been shown in table 2.

The Carbon tetrachloride extract showed mild sensitivity to several gram positive, gram negative bacteria & Fungi (zone of inhibition 9-10 mm). The Methanol extract also showed mild sensitivity to several gram positive, gram negative bacteria & Fungi (zone of inhibition 7-10 mm).and slightly to highly sensitive to fungi (zone of inhibition 8-40 mm). The pet ether extract Crystal (Found after adding CCl_4) showed mild sensitivity to only a gram negative bacteria (*Shigella boydii*) having zone of inhibition 7 mm.

Table 2. *Antimicrobial activity of test samples of Areca catechu*

Micro-organism	Type	Standard Disc	S_1	S_2	S_3	S_4
1. *Bacillius sereus*	Gram +ve	33	9	7	-	-
2. *Bacillius megaterium*	Gram +ve	34	9	7	-	-
3. *Bacillius subtilis*	Gram +ve	34	9	7	-	-
4. *Staphylococcos aureus*	Gram +ve	34	9	7	-	-
5. *Sarcina lutea*	Gram +ve	33	9	7	-	-
6. *Salmonella paratyphi*	Gram -ve	33	10	7	-	-
7. *Salmonella tiphi*	Gram -ve	33	9	8	-	-
8. *Vibrio parahemolyticus*	Gram -ve	33	9	8	-	-
9. *Vibrio mimicus*	Gram -ve	33	9	7	-	-
10. *E.coli*	Gram -ve	34	9	7	-	-
11. *Shigella dysentriae*	Gram -ve	34	9	7	-	-
12. *Pseudomonas aureus*	Gram -ve	33	9	7	-	-
13. *Shigella boydii*	Gram -ve	34	10	10	7	7
14. *Saccharromyces cerevaceae*	Fungi	33	9	7	-	-
15. *Candida albicans*	Fungi	33	9	7	-	-
16. *Aspergillus niger*	Fungi	33	9	7	-	-

4. Conclusion

In the conclusion it can be said that the experiment was helpful for further isolation of natural product as in pain reduction purposes. Most of drugs are not safe when they came from synthetic source but if they are from nature, it becomes better than synthetic. In these investigation demonstrations that, *Mangifera indica* exhibits potential analgesic activities as well as Areca catechu mild antimicrobial activities against several microorganisms. The presence of flavonoid enhances the scope to find out its anti-oxidant property, it seems this would be an ideal agent as anti-inflammatory agent if properly modified. Further investigation may lead to isolation, purification of lead compounds for biological studies.

Abbreviations

S_1: Carbon tetra chloride extract, S_2: Methanol extract, S_3: Pet ether extract, S_4: Crystal (Found after adding CCl4).

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