IN VITRO STUDY ON ALPHA AMYLASE INHIBITORY ACTIVITY OF MURRYA KOENIGII: EFFECT OF EXTRACTION SOLVENTS

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ABSTRACT
Murrya koenigii Linn. Spreng Wettst (M. koenigii L.) is a medicinal plant. In traditional medicine the plant is used to treat leukoderma, piles, stomach problem, kidney pain and blood disorders. Different parts of the plant showed pharmacological activities like anti cancer, anti inflammatory, anti oxidant, anti diabetic, gastro protective, hepato protective, photo-protective anti gastric ulcer, anti microbial etc. The plant also possesses alpha amylase inhibitory activity. Aim of the present work was to check effect of solvent extracts on in vitro alpha amylase inhibitory activity of M. koenigii L. leaves. M. koenigii L. was collected from the local market and identified by the taxonomist. Solvent extractions of the leaves were made separately by using chloroform, petroleum ether, ethanol, isopropanol and hexane. Extracts were separately dried and processed for in vitro alpha amylase inhibitory activity by standard method. Acarbose, an alpha amylase inhibitor, was used as control. Results showed that ethanol extract of M. koenigii L. leaves had maximum alpha amylase inhibitory activity in comparison to that of other solvent extracts. In vitro alpha amylase inhibitory activity of ethanol extract of M. koenigii L. leaves (IC₅₀ value 36.72±1.50 μg/mL) was also comparable to that of acarbose (IC₅₀ value 66.66±1.6 μg/mL). This study therefore indicates uses of ethanol extract of M. koenigii L. leaves in the management of diabetes.
KEYWORDS: Murrya koenigii linn. leaves; solvent extractions; alpha amylase inhibitory activity, acarbose, diabetes.

1. INTRODUCTION
M. koenigii L. (family, Rutaceae), a semi deciduous aromatic herb or small tree with dark green bark., is being used as medicinal plant since long. The plant is found in India, Bangladesh, Nepal, Bhutan, Thailand, Pakistan, Vietnam and Sri Lanka in plenty amount and commonly known as curry leaf as leaves of the plant are often used in curries for flavouring due to their typical flavour. The plant is also known as ‘meehi saag’ in Nepali, ‘bursunga’ in Hindi etc. M. koenigii L. is widely distributed at foothills of Himalayas from Kumaon to Sikkim, Bengal, Assam, middle and lower hill forests up to the height of 5000 ft. February to May is the flowering time of the plant.\[1\]

In traditional medicine M. koenigii L. has many uses. The herb is used for its stomachic and tonic properties. Roots and leaves of the herb are used to treat leukoderma, kidney pain, piles and blood disorders. Burk is used to cure eruptions and poisonous animal bites.\[2\]

Several phytochemicals like murrayacine, murrayazolinol, girinimbioin, 7- methoxy- 3 methyl carbazole- 1,4- quinone, 1- hydroxy -3- methyl carbazole, Me- 2- methoxy carbazole –3- carboxylate, 6, 7-dimethoxy-3-methyl carbazole-1, 4- quinone, murrayazolidine, girinimbine, mukonidine, mahanimbionl, mahanimbilol, 9- formyl –3- methyl carbazole, 9-carbethoxy-3-methyl carbazole, were isolated and characterized from M. koenigii L.\[3\]

Modern researchers showed that different parts of M. koenigii L. possess anti ulcer, antitumor, antimicrobial, anthelmintic, cytotoxic, photoprotective, hepatoprotective, antioxidant, analgesic, anti diarrheal, and anti inflammatory properties.\[4\]

Alpha amylase inhibitory activity of Murrya koenigii is known in literature.\[5\] Aim of the present work was to see effect of extraction solvents on in vitro alpha amylase inhibitory activity of M. koenigii L. leaves.

2. METHODOLOGY
2.1 Collection of plant materials
Leaves of M. koenigii L. were purchased from the local market and authenticated by the taxonomist of the department of Botany of the University of North Bengal, Siliguri, Dist. Darjeeling, West Bengal, India. Voucher specimens of the leaves were deposited in the
department for future references. Leaves of plant were washed thoroughly, shed dried and powered. The powder, used as test drug, was stored desiccated at 4°C until further use.

*Murrya koenigii* Linn. Spreng Wettst

2.2 Solvent extraction

Test drug (50 g) was extracted separately with 500 ml of chloroform, petroleum ether, ethanol, isopropanol and hexane in soxhlet at 37°C for 20 minutes. The extract was filtered and the filtrate was evaporated to dryness in vacuo with rotary evaporator at 40–50°C. This was applied separately for all extracts. Brown masses obtained were used for *in vitro* alpha amylase inhibition assay.

2.3 Alpha amylase inhibition assay

Alpha amylase inhibition assay of the test drug was carried out by the method described by Deguchi *et al.* [6] with slight modifications. 400 μl of 0.1 M sodium phosphate buffer (pH 7.0), 500 μl of 1% starch solution, 10 μg/ml, 20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml and 100 μg/ml of all extracts separately dissolved in DMSO and 50 μl of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/ml) were mixed and incubated at 37 °C for 10 min. 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent was then added. The mixture was kept in a boiling water bath for 5 min and then diluted with 20 ml of distilled water. The absorbance was recorded at 540 nm. Control sample was prepared accordingly without test drug and acted as a negative control. Acarbose was used as positive control. Inhibition capacities of test drug and acarbose were calculated as following:

\[
\text{Inhibition Percentage (\%) = 1 } \frac{\text{DO sample}}{\text{DO control}} \times 100.
\]

All tests were done for five sample replications. IC₅₀ value which is the concentration required to inhibit 50% of alpha amylase activity was calculated in each case.
2.4 Statistical calculation

This was done by SPSS 20. The statistical significance of enzyme inhibitions between test drugs and acarbose, the known inhibitor of alpha amylase, was evaluated with Duncan’s multiple range test (DMRT). 5% was considered to be statistically significant.[7]

3. RESULTS

Results are summarized in Table -1. Acarbose, standard alpha amylase inhibitor, in the concentrations of 10, 20, 40, 60, 80 and 100 μg/ml showed 19.18±1.1, 23.23±1.0, 35.34±1.2, 45.87±1.3, 56.28±1.8 and 59.55±1.9 respectively percent of inhibitions in alpha amylase activity with IC$_{50}$ value 66.66±1.6 μg/ml. Chloroform extract of _M. koenigii_ L. leaves, on the other hand, showed activity 11.25±0.2, 19.37±1.0, 37.44±1.1, 41.56±1.2, 55.71±1.6 and 65.65±1.8 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 μg/ml respectively. IC$_{50}$ value came 73.17±1.8 μg/ml.

Petroleum ether extract of _M. koenigii_ L. leaves in the concentrations of 10, 20, 40, 60, 80 and 100 μg/ml showed 20.34±0.9, 25.78±1.1, 41.06±1.3, 50.35±1.5, 59.48±1.7 and 70.76±2.0 percent of inhibitions in alpha amylase activity respectively with IC$_{50}$ value 58.72±1.2 μg/ml.

Table 1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and different solvent extracts of _M. koenigii_ L. leaves.

<table>
<thead>
<tr>
<th>Drug/solvent extract</th>
<th>Concentration (μg/ml)</th>
<th>% of inhibition</th>
<th>IC$_{50}$ Value (μg/ml)</th>
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</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>10</td>
<td>19.18±1.1</td>
<td>66.66±1.6</td>
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<td>20</td>
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<td>45.87±1.3</td>
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<td>56.28±1.8</td>
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<td>100</td>
<td>59.55±1.9</td>
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<td>Chloroform extract of <em>M. koenigii</em> L. leaves</td>
<td>10</td>
<td>11.25±0.2</td>
<td>73.17±1.8</td>
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<td>20</td>
<td>19.37±1.0</td>
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<td>Petroleum ether extract of <em>M. koenigii</em> L. le</td>
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<td>20.34±0.9</td>
<td>58.72±1.2</td>
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<td>leaves</td>
<td>20</td>
<td>25.78±1.1</td>
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<td>41.06±1.3</td>
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<td>59.48±1.7</td>
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<td>100</td>
<td>70.76±2.0</td>
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<tr>
<td>Ethanol extract of <em>M. koenigii</em> L. leaves</td>
<td>10</td>
<td>28.12±1.0</td>
<td>36.72±1.50*</td>
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<td></td>
<td>20</td>
<td>39.37±1.2</td>
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Ethanol extract of *M. koenigii* L. leaves, however, showed 28.12±1.0, 39.37±1.2, 67.56±1.5, 71.61±1.6, 75.58±2.0 and 85.44±2.1 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 μg/ml respectively. IC50 value was 36.72±1.50 μg/ml.

Isopropanol extract of *M. koenigii* L. leaves in the concentrations of 10, 20, 40, 60, 80 and 100 μg/ml showed 23.23±1.0, 29.41±1.2, 47.55±1.4, 58.62±1.6, 65.75±1.7 and 78.82±1.9 percent of inhibitions in alpha amylase activity respectively with IC50 value 50.57±1.1 μg/ml.

Hexane extract of *M. koenigii* L. leaves, on the other hand, showed 15.87±1.0, 22.46±1.1, 40.39±1.2, 49.27±1.4, 57.66±1.4 and 69.71±1.6 percent of inhibitions respectively in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 μg/ml. IC50 value came 61.57±1.3 μg/ml.

### 4. DISCUSSION

Diabetes mellitus, a chronic metabolic non-communicable disease, took several lives till today. Only in 2015 diabetes mellitus was the cause of death for 5 million people worldwide.[8] Diabetes mellitus is increasing rapidly. There were approximately 108 million diabetic patients in the world in 1980 but in 2014 the number has been increased to 422 million. Presently highest population of diabetics are found in China, India, USA, Brazil, Mexico and Indonesia. In India diabetes is increasing so fast that the number of adults with diabetes is expected to reach 87 million by the year 2030.
Diabetes mellitus particularly Type – 2 diabetes mellitus is characterized by postprandial hyperglycemia. One of the therapeutic approaches, therefore, is to reduce postprandial hyperglycemia. This can be done by inhibiting carbohydrate splitting enzymes. One such enzyme is alpha amylase which hydrolyses complex carbohydrates of food to free sugars. Inhibition of alpha amylase reduces hydrolysis of complex carbohydrate thereby postprandial hyperglycemia is checked. Acarbose, one alpha amylase inhibitor, has already been included in the list of drugs of Type - 2 diabetes mellitus. In this context medicinal plants were also investigated for alpha amylase inhibitory activity and many plants were found having alpha amylase inhibitory activity.

The present work showed alpha amylase inhibitory activity of *M. koenigii* L. leaves in *in vitro* experiments. Ethanol, isopropanol, petroleum ether, hexane and chloroform extracts of *M. koenigii* L. leaves in all concentrations exerted *in vitro* alpha amylase inhibitory activity which was comparable to that of acarbose, standard alpha amylase inhibitor (Figure – 1). Maximum activity, however, was noted in ethanol extract. This is evident when examined alpha amylase inhibitory activity in all doses (10, 20, 40, 60, 80 and 100 μg/ml) of ethanol extract and the same doses of acarbose as well as chloroform, petroleum ether, isopropanol and hexane extracts (Figure – 2). This is further evident when examined IC$_{50}$ value in alpha amylase inhibitory activity of ethanol extract of *M. koenigii* L. leaves (36.72±1.50 μg/ml) and the IC$_{50}$ values of acarbose (66.66±1.6 μg/ml), isopropanol (50.57±1.1 μg/ml), petroleum ether (58.72±1.2 μg/ml), hexane (61.57±1.3 μg/ml) and chloroform (73.17±1.8 μg/ml) extracts of *M. koenigii* L. leaves (Figure – 3).

![Figure 1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and different solvent extracts of *M. koenigii* L. leaves.](image-url)
Figure – 2: Alpha amylase inhibitory activity in different doses of acarbose and various solvent extracts of *M. koenigii* L. leaves in the same doses.

Figure – 3: IC$_{50}$ values (μg/ml) in alpha amylase inhibitory activity of acarbose and different solvent extracts of *M. koenigii* L. leaves.

Dineshkumar *et al.*, however, observed that petroleum ether extract of *M. koenigii* L. had maximum inhibition (%) on alpha amylase activity$^5$ while Ponnusami *et al.* noted that isopropanol extract from *M. koenigii* leaves had maximum inhibition (%) on alpha amylase activity.$^{12}$ The present study therefore advocates use of ethanol extract of *M. koenigii* L. leaves in Type – 2 diabetes mellitus to keep postprandial blood sugar under control.

It is known that biological activity of medicinal plants depends on season.$^{13}$ We are now working on seasonal variation in alpha amylase inhibitory activity of *M. koenigii* L. leaves.
5. CONCLUSION

Based on the present work compound responsible for alpha amylase inhibitory activity may be isolated from the ethanol extract of *M. koenigii* L. leaves which, in turn, may be used in future as drug for diabetes.

REFERENCES


12. Ponnusamy Sudha, Ravindran Remya, Zinjarde Smita, Bhargava Shobha, Ravi Kumar Ameeta. Evaluation of Traditional Indian Antidiabetic Medicinal Plants for Human Pancreatic Amylase Inhibitory Effect In Vitro. Evidence-Based Complementary and Alternative Medicine, 2011; 1-10. (On line publication)