ISOLATION OF A COMPOUND FROM MURRYA KOENIGII (LINN.) SPRENG WETTST LEAVES AND STUDIES ON ITS IN VITRO ANTI OXIDANT STATUS

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ABSTRACT

Murrya koenigii Linn. Spreng Wettst (M. koenigii L.) is widely used in Indian cookery for centuries as it promotes appetite and digestion. The plant has several pharmacological activities including anti oxidant property. The aim of the present work is to isolate a compound from M. koenigii L. leaves and to study its anti oxidant property. M. koenigii L. leaves were collected, identified by the taxonomist, washed thoroughly, shade dried and powdered. Principles of standard isolation procedures of chemical compounds from plant sources were maintained to isolate a compound from the leaves. In vitro anti oxidant activity of the isolated compound was measured by superoxide anion generation with the help of xanthine-xanthine oxidase assay and with linoleic acid peroxidation assay as well as by DPPH photometric assay. From the leaves of M. koenigii L. a compound was isolated. The compound showed significant in vitro anti oxidant effect. The compound may, therefore, be used as natural anti oxidant.

KEYWORDS: Murrya koenigii L., Isolation of a compound, anti oxidant property.

1. INTRODUCTION

M. koenigii L. (family, Rutaceae) is a medicinal plant widely distributed at foothills of Himalayas from Kumaon to Sikkim, Bengal, Assam, middle and lower hill forests up to the height of 5000 ft. It is a small tree with dark green bark, often cultivated. February to May is the flowering time of the plant. The plant has several names. In Nepali it is called ‘meehi saag’, in Hindi ‘bursunga’ and in English it is known as ‘curry leaf tree’. Leaves of the plant are often used in curries for flavouring due to their typical flavour.

In traditional medicine M. koenigii L. has several uses. Leaves and roots are used to treat kidney pain, piles, leukodermia and blood disorders. Burk is used to cure eruptions, poisonous animal bites etc. The plant is also used for its stomachic and tonic properties.¹,² Several bio active compounds have been isolated and characterized from this plant. These bioactive compounds possess antidiarrheal, hepatoprotective, antimicrobial, anthelmintic, anti ulcer, antioxidant, analgesic, cytotoxic, antitumor and anti inflammatory properties.³ We also reported anti gastric ulcer activity of M. koenigii L. leaves in albino rats.⁴,⁵ Anti oxidative property of M. koenigii L. leaves is well documented in literature.⁶,⁷

The present work was aimed to isolate a compound from the leaves of M. koenigii L. and to study its anti oxidant status.

2. METHODOLOGY

2.1 Collection of plant materials

Leaves of M. koenigii L. were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, west Bengal, India randomly. Leaves were authenticated by the taxonomist of the department of Botany of the said University. Voucher specimens of the leaves were deposited in the department for future references. Leaves of plants were washed thoroughly, shade dried and powdered. The powder was used for isolation work.
2.2 Isolation work
This was done by the following scheme. Principles of standard isolation procedures of chemical compounds from plant sources were followed.\textsuperscript{[8–11]}

2.3 Antioxidant assays
Antioxidant activity of the isolated compound was assayed by superoxide anion generation by xanthine-
/xanthine oxidase assay \textsuperscript{[12]} linoleic acid peroxidation assay \textsuperscript{[13]} and by DPPH photometric assay \textsuperscript{[14]}.

2.4 Chemicals
Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab, India and from Merck, Germany.

2.5 Diagrammatic scheme for isolation of a compound from leaves of \textit{M. koenigii} L.

\begin{itemize}
\item powdered leaves of \textit{M. koenigii} L. (50 g)
\item extracted with 500 ml of 80 : 20 (v/v) Petroleum ether : n-hexane mixture for 15 min at 37°C in a Soxhlet apparatus. It was then centrifuged. Supernatant collected and evaporated to dryness.
\item refluxed with 50 ml of 1(N) HCL for 10 min on a water bath at 100 °C. It was then cooled and centrifuged. Supernatant was evaporated to dryness.
\item extracted with 50 ml ethyl acetate on a rotary shaker for 10 min. It was then centrifuged. Supernatant was evaporated to dryness.
\item extracted with 15 ml methanol for 10 min. It was then filtered. With filtrate alumina column chromatography was performed. Elution was done by acetone – methanol mixture (60:40 v/v).
\item eluent of active fourth band was evaporated to dryness. The dry mass was extracted with 15 ml ethanol for 10 min. It was then filtered. With filtrate polyamide column chromatography was done. Elution was done by chloroform : ethanol mixture (60:40 v/v).
\item eluent of active second band was evaporated to dryness. The dry mass was extracted with 10 ml acetone for 10 min. It was then filtered and the filtrate was subjected to silica gel column chromatography using silica gel G as adsorbent. Elution was done by acetone, chloroform mixture (60:40 v/v).
\end{itemize}
CRYSTALLIZATION

Eluent of the active third band obtained from the above step was evaporated to dryness. Repeated crystallization was done from ethyl formate–formic acid (60:40, v/v) mixture.

Crystals obtained (4.6 mg)

3. RESULTS

3.1 Isolation of compound

One compound was isolated from the leaves of *M. koenigii* L.

3.2 Anti oxidant status of the isolated compound

*In vitro* antioxidant activity of the compound, isolated from *M. koenigii* L. leaves, through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay was given in Figure. 1

*In vitro* antioxidant activity of the compound isolated from *M. koenigii* L. leaves through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay came 94, 95 and 97 respectively in terms of percent inhibition. This anti oxidant activity of the isolated compound was compared with quercetin, a known anti oxidant agent. Under the same condition *in vitro* antioxidant activity of quercetin through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay came 100, 98 and 100 respectively in terms of percent inhibition. Both the isolate compound and quercetin were used in the dose of 100 μg / ml as per our earlier report. [15]

Table 1: *In vitro* antioxidant activity of the compound, isolated from *M. koenigii* L. leaves, through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay.

<table>
<thead>
<tr>
<th>Category 1: xanthine-/xanthine oxidase assay</th>
<th>Category 2: linoleic acid peroxidation assay</th>
<th>Category 3: DPPH photometric assay</th>
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</thead>
<tbody>
<tr>
<td>■ Quercetin, 100 μg / ml</td>
<td>■ Isolated compound from <em>M. koenigii</em> L. leaves, 100 μg / ml</td>
<td>Results were mean of five experiments.</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Free radicals are generated in human body through aerobic respiration.[16] They are also generated from exogenous sources. These free radicals cause oxidative stress which is responsible for induction of many chronic and degenerative diseases like diabetes, cancer, atherosclerosis, ageing, immunosuppression, ischemic heart disease etc. [17]

Antioxidants are those substances which break free radical chain reaction minimizing oxidative stress. There is anti oxidant defense mechanism in human body but still there is demand for more anti oxidant compounds. To fulfil the demand synthetic anti oxidant compounds like butylated hydroxyanisole and butylated hydroxytoluene are available. But they are not safe. Their toxicity is a matter of concern. It is often claimed that these synthetic anti oxidants have many side effects including carcinogenicity.[18] Therefore, there are high demands for naturally occurring anti oxidants. Continuous search is going on for natural anti oxidant compounds which is even extended in the field of medicinal plants also.[19]

In the present study one anti oxidant compound has been isolated from the leaves of *M. koenigii* L. Anti oxidant activity of the compound was found to be more or less same to that of quercetin, a known synthetic anti oxidant compound. Isolated compound needs characterization. Work is now going on in this direction.
5. CONCLUSION
In this study compound isolated from the leaves of *M. koenigii* L. may be used as natural anti oxidant in future.

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Conflict of interest: There is no conflict of interest.

REFERENCES