IN-VITRO ANTI OXIDANT ACTIVITY OF CHROMATOGRAPHICALLY SEPARATED FRACTIONS FROM SYZYGIUM CUMINI L. LEAVES

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ABSTRACT

Aim: Anti-oxidant activity of leaves of Syzygium cumini Linn. (S. cumini L.), a tropical fruit tree of great economic importance possessing several pharmacological properties, is known in literature. We have also found in vitro anti-oxidant activity of methanol extract of S. cumini L. leaves. An attempt was, therefore, made to isolate the anti-oxidant compound from the leaves of M. spicata L. Material & Methods: Leaves of S. cumini L. were processed for isolation of anti-oxidant compound. Solvent extraction, acid hydrolysis, solvent treatment followed by chromatographic experiment were done. Separated fractions in chromatographic experiments were assayed for in vitro anti-oxidant activity by superoxide anion generation with the help of xanthine-xanthine assay, linoleic acid peroxidation assay and DPPH photometric assay. Total phenol, flavonoids, ascorbic acid and carotenoid contents in the separated fractions were also determined. Results: Six fractions were separated in the chromatographic experiment. Fraction number four showed highest anti-oxidant activity which had relation with presence of high amounts of total phenol, flavonoid, ascorbic acid and carotenoids in the fraction. Conclusion: The fraction may be processed further for isolation of anti-oxidant compound.

Keywords: Syzygium cumini Linn., chromatographic separation, anti-oxidant activity, total phenol, flavonoid, ascorbic acid, carotenoids.

INTRODUCTION

In human body free radicals are generated through aerobic respiration or from exogenous sources [1]. These free radicals namely hydroxyl radical, superoxide anion, hydrogen peroxide etc. also known as reactive oxygen species (ROS) cause oxidative stress and may react with biological molecules like proteins, lipid and deoxy ribonucleic acid to produce molecular alterations which, in turn, are associated with various degenerative human diseases such as, diabetes, cancer, Alzheimer’s disease, Parkinson’s disease, arteriosclerosis, aging, arthritis, mongolism, immune deficiency diseases, asthma etc. [2] Antioxidative defense mechanisms are the effective paths to eliminate and diminish the action of free radicals. Human body has antioxidative defense mechanism. Still it needs more antioxidants. To cope up the situation anti-oxidants were synthesized chemically. Butylated hydroxyanisole and butylated hydroxytoluene are examples of synthetic antioxidants. They are commercially available but their toxicity is a matter of concern, It is reported that these synthetic antioxidants may develop several diseases including cancer [3]. Therefore there was continuous search for natural anti-oxidants in different sources and the search has even extended to plant kingdom.

S. cumini L. is a tropical plant of great economic importance. Almost all parts of the plant are used for various purposes. Wood due to its resistant power towards water and termitic is used for preparation of water and termitic resistance materials. Leaves of the plant are used as fodder. Oil obtained after distillation of S. cumini L. leaves due to its fragrance is used in preparation of soaps and perfumes. Flowers are used in apiculture for production of high quality honey. Fruit pulp is used to make juice, vinegar, jams, jellies and puddings. Fruits are also used to make wine [4].

All parts of S. cumini L. also possess medicinal properties. Bark, seed and pulp are efficacious for diabetes. Leaf has anti-bacterial, anti-diabetic, anti-viral, anti-allergic, anti-oxidant and anti DNA damage activities. Seeds exert anti-gastric ulcer and anti-inflammatory activity. Fruits are anti-hyper lipidemic, possess anti- cancer property [5]. During phytochemical studies compounds like ß-sitosterol-D-glucoside, quecertin, myricetin, betulinic acid, ß-sitosterol, astragalin kaempferol-3-o-glucoside, tryidelin, epi-friedelanol , Eugenin, gallic acid, n-hepatosane, n-nonacosane, acid soxalic, citric acid, glycolic acids, sitosterol, betulinic acid, crategolic (maslinic) acid, n-hentriacontane ,n-octacosanol, n-triacontanol , kaempferol – 3-ß-D-glucuronopyranoside, ellagittannin, nilocitin, myricetin 3-ß-D-glucuronopyranoside and aminoacids like glycine, alanine, oleicnolic acid, eragteolic acid (maslinic acid ), quecetin, kaempferol and myricetin flavonoids – isouqueritrin are found present in different parts of S. cumini L. [6,7]. S. cumini L. leaf is known for its anti-oxidant property [8,9]. Recently we confirmed anti-oxidant activity of methanolic extract of S. cumini L. leaf. Results are under communication. In present study attempts have been made to isolate antioxidant compounds from S. cumini L. leaf.

MATERIAL AND METHOD

Collection of plant materials
S. cumini L. leaves were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Siliguri (26°41.30.9984 N, 88°27.4.5756 E, elevation, 410 ft), Dist. Darjeeling, West Bengal, sometimes in the month of July – August 2016. Leaves were authenticated by the taxonomist of the department of Botany of the University of North Bengal, Siliguri. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references.

![Syzygium cumini L. leaves](image-url)
Test drug
Leaves of *S. cumini* L. were washed thoroughly, shed dried and powered. The powder, used as test drug, was stored desiccated at 4 °C until further use.

Isolation process
This was done by the following steps. Principles of standard isolation procedures of chemical compounds from plant sources were followed [10-13].

**Steps towards isolation of antioxidant compound from leaves of S. cumini L.**
Powdered leaves of *S. cumini* L. (100 g)

![](image)

Six bands were separated

**Antioxidant assays**
Antioxidant activity of the chromatographically separated fractions was assayed by superoxide anion generation by xanthine-xanthine oxidase assay [14] linoleic acid peroxidation assay [15] and DPPH photometric assay [16].

**Flavonoids content**
Flavonoids content of chromatographically separated fractions was determined using Aluminum chloride colorimetric method [17].

**Total phenols content**
Total phenols content of chromatographically separated fractions was determined by Folin Ciocalteu reagent [16].

**Ascorbic acid content**
Ascorbic acid content of chromatographically separated fractions was determined by the method of Cakmak and Marschner [19].

**Carotenoids content**
Total carotenoids of chromatographically separated fractions were determined by the method of Jensen [20].

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

**Statistical analysis**
The statistical significance between antioxidant activity values of the powdered leaves of *S. cumini* L. was evaluated with a Duncan’s multiple range test (DMRT). 5% was considered to be statistically significant [21].

**RESULTS**
Antioxidant activity of chromatographically separated fractions obtained during isolation of anti-oxidant compound from powdered leaves of *S. cumini* L. by superoxide anion generation by xanthine-xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay is given in Table 1.

**Table 1:** Inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by chromatographically separated fractions obtained during isolation of anti oxidant compound from powdered leaves of *S. cumini* L.

<table>
<thead>
<tr>
<th>Chromatographically separated fractions of powdered leaves of <em>S. cumini</em> L</th>
<th>Xanthine oxidase (% inhibition)</th>
<th>Linoleic acid peroxidation (% inhibition)</th>
<th>DPPH (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction : 1</td>
<td>34 ± 0.3</td>
<td>29 ± 0.3</td>
<td>27 ± 0.2</td>
</tr>
<tr>
<td>Fraction : 2</td>
<td>38 ± 0.4</td>
<td>25 ± 0.2</td>
<td>33 ± 0.2</td>
</tr>
<tr>
<td>Fraction : 3</td>
<td>50 ± 0.4</td>
<td>40 ± 0.5</td>
<td>39 ± 0.4</td>
</tr>
<tr>
<td>Fraction : 4</td>
<td>98 ± 0.6*</td>
<td>92 ± 0.6*</td>
<td>96 ± 0.5*</td>
</tr>
<tr>
<td>Fraction : 5</td>
<td>42 ± 0.5</td>
<td>41 ± 0.4</td>
<td>48 ± 0.2</td>
</tr>
<tr>
<td>Fraction : 6</td>
<td>37 ± 0.4</td>
<td>37 ± 0.5</td>
<td>36 ± 0.4</td>
</tr>
<tr>
<td>Quercetin</td>
<td>100 ± 0.2</td>
<td>95 ± 0.2</td>
<td>99 ± 0.2</td>
</tr>
</tbody>
</table>

Concentration used : 100 μg / ml [22]. Results were a mean of triplicate experiments. Significant

Chromatographically separated all fractions obtained during isolation of anti oxidant compound from powdered leaves of *S. cumini* L. had in vitro anti-oxidant activity but maximum activity was observed for fraction - 4. Inhibition in xanthine oxidase, linoleic acid peroxidation and DPPH inhibitions respectively was found 98%, 92% and 96% respectively with fraction – 4. Results were statistically significant in comparison to that of other fractions and comparable with quercetin, a standard anti-oxidant. In case of quercetin inhibition in xanthine oxidase, linoleic acid peroxidation and DPPH inhibitions came 100%, 95% and 99% respectively.

Amounts of total phenol, flavonoids, ascorbic acid and carotenoids present in chromatographically separated fractions obtained during isolation of anti oxidant compound from powdered leaves of *S. cumini* L. are given in table – 2.

**Table 2:** Total phenol, flavonoids, ascorbic acid and carotenoids contents of chromatographically separated fractions obtained during isolation of anti oxidant compound from powdered leaves of *S. cumini* L.

<table>
<thead>
<tr>
<th>Chromatographically separated fractions of powdered leaves of <em>S. cumini</em> L</th>
<th>Total phenol content (mg/g dry wt)</th>
<th>Total flavonoid content (mg/g dry wt)</th>
<th>Ascorbic acid content (mg/g dry wt)</th>
<th>Carotenoids content (mg/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction : 1</td>
<td>20.6 ± 0.2</td>
<td>19.7 ± 0.3</td>
<td>8.6 ± 0.2</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>Fraction : 2</td>
<td>18.9 ± 0.4</td>
<td>15.3 ± 0.3</td>
<td>5.2 ± 0.2</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>Fraction : 3</td>
<td>30.3 ± 0.5</td>
<td>56.1 ± 0.5</td>
<td>13.8±0.3</td>
<td>14.7 ± 0.2</td>
</tr>
<tr>
<td>Fraction : 4</td>
<td>47.8 ± 0.4*</td>
<td>86.4 ± 0.6*</td>
<td>24.5±4*</td>
<td>23.2±0.3*</td>
</tr>
<tr>
<td>Fraction : 5</td>
<td>35.9 ± 0.3</td>
<td>52.7 ± 0.5</td>
<td>15.5±0.3</td>
<td>16.5 ± 0.3</td>
</tr>
<tr>
<td>Fraction : 6</td>
<td>27.1 ± 0.3</td>
<td>32.5 ± 0.3</td>
<td>8.8 ± 0.1</td>
<td>10.3 ± 0.2</td>
</tr>
</tbody>
</table>

Results were a mean of triplicate experiments. Significant

Table 2 shows that chromatographically separated fraction - 4 contained maximum amount of total phenol (47.8 ± 0.4 mg/g dry weight), total flavonoids (86.4 ± 0.6 mg/g dry weight), ascorbic acid (24.5 ± 0.4 mg/g dry weight) and carotenoids (23.2 ± 0.3).
mg/dry weight). Other fractions obtained during chromatographic separation were found contained less amounts of total phenol, flavonoids, ascorbic acid and carotenoids.

**DISCUSSION**

Since long researchers were searching natural antioxidants in various sources. Medicinal plants were also investigated for their anti-oxidant activity. Balasundram et al. isolated phenolic compounds from various plants and noted their anti-oxidant potentialities. [23] Thereafter series of phenolic compounds including caffeic, quinic, and p-coumaric acids were isolated from different plants which showed their anti-oxidant activities. [24] Kahkonen et al. examined antioxidant activity of plant extracts containing phenolic compounds and inferred that phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals. [25] Lai et al. studied antioxidative activities of Hsiantsao (Mesona procumbens.Hems.l.) leaf gum and concluded that antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases.[26]

In the present study during isolation of anti-oxidant compound from *S. cumini* L. leaves chromatographic experiments were done after solvent extract and acid hydrolysis. Six fractions were separated in Silica gel – G column chromatography. The fourth fraction showed maximum anti-oxidant activity as measured by inhibition in xanthine oxidase, linoleic acid peroxidation and DPPH inhibitions (Figure-1). The activity was comparable to that of quercetin, the standard synthetic anti-oxidant compound.

![Fig. 1: Showing inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by chromatographically separated fractions obtained during isolation of anti-oxidant compound from powdered leaves of *S. cumini* L.](image1)

**CONCLUSION**

In the present experiment it was shown that fraction – four obtained after chromatographic separation during isolation of anti-oxidant compound from powdered leaves of *S. cumini* L showed maximum in vitro anti-oxidant activity. Fraction – four, therefore, may be used as natural anti-oxidant and further be studied for isolation of the pure natural anti-oxidant compound(s).

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

Nil.

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