Evaluation of antidiabetic phytochemicals in Syzygium cumini (L.) Skeels (Family: Myrtaceae)

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ABSTRACT
Traditionally the leaves of Syzygium cumini (Myrtaceae) are widely used for treating diabetes. The present study was carried out to identify the putative antidiabetic constituents from the S. cumini leaves. From the NMR data four different compounds, Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, β-sitosterol were identified from n-hexane fraction of plant extract. These compounds have potential antidiabetic activities which support the traditional use of the leaves as being remedy for treating diabetes.

INTRODUCTION

Syzygium cumini (L.) Skeels, a polyembryonic species (family-Myrtaceae), is a tropical fruit tree of great economic importance (Chase et al., 2009). It is a large evergreen tree up to 30 meters height and girth of 3.6 meters with a bole up to 15 meters. The fruit is commonly known as kaloum (Bangla), Jamun (Hindi), java plum, black plum and Indian blackberry. It is widely distributed forest tree of India, Bangladesh, Sri Lanka, Malaysia and Australia which is also cultivated for its edible fruits. The tree was introduced from India and tropical Asia to southern Africa for its edible and attractive fruits. S. cumini has been widely used for the treatment of various diseases in traditional and folk medicine. Unani system of medicine describes the use of the plant in liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head (Ayyanar et al., 2012). Various studies reported that, the bark of the plant has various properties like astringent, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial activity (Saravanan and Pari, 2008). The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection (Saravanan and Pari, 2008). The leaves are antibacterial and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomalalgia, fever, gastropathy, strangury, dermopathy and to inhibit blood discharge in the feces (Ravi et al., 2005; Sagrawat et al., 2006; Gowri and Vasantha, 2010). The barks, leaves and seeds extracts of S. Cumini have also been reported to possess anti-inflammatory antibacterial and antidiarrheal effects (Indira and Mohan, 1992; Chaudhuri et al., 1990; Bhuiyan et al., 1996). Powdered seeds are used as a remedy in diabetes and in menorrhagia (Sharma and Mehta, 1969). It has been also showed before that the leaf, bark, stem and pulp of S. cumini plants possess potent antidiabetic activity (Chaudhary et al., 2012; Kumar et al., 2008; Leelavinothan and Saravanan, 2006; Farswana et al., 2009; Bopp et al., 2009).
The major phytoconstituents are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components (Martinez and Del Valle, 1981; Wealth of India, 1976). Preliminary phytochemical analysis also showed the presence of Phenols, Terpenoids, Tannins, Saponins, Phytoesters, Carbohydrates, Flavonoids, Amino acids in stem bark of S. cumini (Kuncha et al., 2012). Previous investigations also revealed the bark of S. cumini contains butulinic acid, β-sitosterol, friedelin, epi-friedelanol (Wealth of India, 1982). It also contains new esters of epi-friedelanol (eugenin), D-glucoside, kaempferol-3-O-glucoside, quercetin, myricetin, astragalin and gallic acid (Sengupta and Das, 1965; Bhargava et al., 1974; Williamsen et al., 2002). The present study was designed to investigate the phytochemical bioactive compounds of the methanolic extract of S. cumini leaves to establish its antidiabetic activity.

MATERIALS AND METHODS

Collection and Identification of plant materials

Fresh plant leaves of Syzygium cumini was collected from Savar, Dhaka, Bangladesh in November, 2011. This plant was identified by Bangladesh National Herbarium (DACB). The collection number for the plant was HM09 and accession number was 423498.

Preparation of plant extract

The collected leaves of Syzygium cumini were washed generally and then kept for drying in the sun for seven days. The plant materials were then oven dried for 24 hours at low temperature. Powdered material of S. cumini leaves was macerated with methanol in round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness (45°C) under reduced pressure by rotary evaporator. The percentage yield of the extract was calculated by using the formula below:

\[ \text{% yield} = \left( \frac{\text{weight of extract}}{\text{weight of plant material}} \right) \times 100\% \]

Mother solution was prepared by mixing 15 gm of methanolic extract by triturating with 270 ml of methanol containing 30 ml distilled water. This solution was partitioned successfully by four solvents of different polarity. The mother solution was taken in a separating funnel. 100 ml of n-hexane was added here and the funnel was shaken and kept undisturbed. Then the organic portion was collected and repeated thrice. Carbon tetrachloride (CCl4) and dichloromethane (CH2Cl2) extract was collected with the help of aqueous mother fraction adding 38 ml and 48 ml distilled water respectively keeping the other procedure unchanged. Finally n-hexane, CCl4, CH2Cl2 and aqueous extract were obtained.

Detection of chemical compounds by NMR spectroscopy

In order to isolate different types of compounds n-hexane soluble fraction was subjected to TLC screening. This revealed a considerable number of compounds and required further fractionation. Sephadex was soaked in a mixed solvent of n-hexane: Dichloromethane: Methanol at the ration of 2:5:1 for swelling. Then slurry found from the extraction of the plant was added into glass column. The previous solvent mixture was used as initial mobile phase. The column was eluted with the same solvent mixture and finally washed with dichloromethane and methanol mixture with increasing polarity. Different fractions were collected in 28 test tubes and these were rendered for evaporation to dryness. 1 to 14, 15 to 22, 23 to 26, 27 to 28 test tubes had solvent systems n-hexane: Dichloromethane: Methanol (20:50:10), Dichloromethane: Methanol (90:10), Dichloromethane: Methanol (50:50) and methanol (100%) respectively. These column fractions were screened by TLC under UV light after spraying with vanillin-sulfuric acid reagent. Fractions having significant result were selected for further investigations with small column and suitable solvent systems. Crystal found in the end was analyzed by NMR for detection of isolated compound. 1H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the spectra were referenced to the residual non-deuterated solvent (CDCl3) signals. Column chromatography (CC) was conducted over (Merck, Germany) sephadex (LH-20). Spot on TLC plates were visualized under UV light (254 and 366 nm) after spraying with vanillin-sulfuric acid, followed by heating at 110°C for 5-10 minutes.

RESULTS

Plant extraction

The yield of the methanolic extract of leaves of S. cumuni was 9.87 % (w/w) dry matter.

Chemical Compounds

Test tube 6, 7, 8 and 9 were selected for further investigation because of their significant result . The column elution was separated in 22 beakers with the help of a small column and mobile phase consisting of ethyl acetate and hexane in 14:86 ratios. These were kept at room temperature covered with aluminum foil to dryness. White crystals were observed in different beakers after four days.. These crystals were then analyzed by NMR. Different fractions contained different compounds which are presented in Table 1. The structure of the compounds found after analyzing in NMR is presented in Figure 1. 1H NMR spectrum of compound 1 showed a double doublet (J = 11.5, 5.03 Hz) of one proton intensity at δ 3.21 ppm, typical of an oxymethine proton at C-3 of a triterpene. The splitting pattern of this proton confirmed the β orientation of the C-3 oxygenated substituent. The spectrum also displayed two singlets at δ 4.68 and 4.56 ppm (1H each) assignable to the vinylic protons at C-29. The 1H NMR spectrum showed seven singlets at δ 0.95, 0.79, 0.83, 1.02, 0.93, 0.799 and 1.68 ppm (3H each) assignable to methyl group protons at C-4 (H2-23, H2-24), C-10 (H2-25), C-8 (H2-26), C-14 (H-27), C-17 (H-28) and C-20 (H2-30), respectively. By comparing the 1H NMR data with previously published data, compound 1 was identified as lupeol (Aratanechemuge et al.,
The identity of 1 was further substantiated by co-TLC with an authentic sample of lupeol. The $^1$H NMR spectra of compounds 2 and 3 readily demonstrated the steroidal nature of these compounds. The spectral data of compounds 2 and 3 were superimposable to the $^1$H NMR spectral data published for $\beta$-sitosterol and stigmasterol (Morales et al., 2003; Kolak et al., 2005). Additionally, thin layer chromatographic analysis of 2 and 3 with authentic samples of $\beta$-sitosterol and stigmasterol, respectively, also confirmed their identity. $^1$H NMR spectrum of compound 4 displayed a proton broad singlet at $\delta$ 5.18 which indicates the presence of olefinic proton. Eight singlets each of three proton intensity at 1.12, 1.06, 1.02, 1.00, 0.97, 0.93, 0.87, 0.84 ppm (3H each) assignable to methyl group protons at C-4 (H$_3$-23, H$_3$-24), C-10 (H$_3$-25), C-8 (H$_3$-26), C-14 (H$_3$-27), C-17 (H$_3$-28) and C-20 (H$_3$-29, H$_3$-30) respectively. By comparing the $^1$H NMR data with previously published data and compound 4 was identified as 12-oleanen-3-ol-3$\beta$-acetate (Krishnaswamy et al., 1975). Spectral analysis is shown in Table 2.

**Table 1:** Constituents in different Solvent Systems

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Developing solvent system</th>
<th>Name of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-12</td>
<td>Ethyl acetate: Hexane = 14:86</td>
<td>12-oleanen-3-ol-3$\beta$-acetate</td>
</tr>
<tr>
<td>16-17</td>
<td>Ethyl acetate: Hexane = 14:86</td>
<td>12-oleanen-3-ol-3$\beta$-acetate</td>
</tr>
<tr>
<td>18-19</td>
<td>Ethyl acetate: Hexane = 14:86</td>
<td>Stigmasterol</td>
</tr>
<tr>
<td>20</td>
<td>Ethyl acetate: Hexane = 14:86</td>
<td>Stigmasterol</td>
</tr>
<tr>
<td>21-22</td>
<td>Ethyl acetate: Hexane = 14:86</td>
<td>Stigmasterol, $\beta$-sitosterol</td>
</tr>
<tr>
<td>Rest of 16</td>
<td>Ethyl acetate: Hexane = 14:86</td>
<td>Lupeol</td>
</tr>
</tbody>
</table>

**Fig. 1:** The structures of the Compounds found in *S. cumuli*.
DISCUSSION

NMR data indicated the presence of Lupeol, 12-olean-3-ol-3β-acetate, Stigmasterol, β-sitosterol in n-hexane portion. According to Panda et al. (2009) stigmasterol has significant effect on lowering serum glucose concentration with a concomitant increase in insulin level indicating it’s hypoglycemic and insulin stimulatory activity. Further, Batta et al. (2006) found that this plant sterol has been found to compete with cholesterol for intestinal absorption and thus results in lowering the plasma concentration of cholesterol level. It was reported that stigmasterol suppress cholesterol biosynthesis via inhibition of sterol Δ24-reductase in human Caco-2 and HL-60 cell lines thus reducing hepatic cholesterol. Again, study of Li et al. (2004) and Gupta et al. (2011) reported that beta-sitosterol has antidiabetic activity though there was no evidence about the exact mechanism. However, Jamaluddin et al. (1994) showed that the structure elucidation of the hypoglycaemic fractions proved the presence of stigmasterol along with beta-sitosterol. When these constituents were tested individually they showed no activity which concluded that synergism between these two is necessary to produce the antidiabetic effect. Lupeol, a phytoconstituent is known to suppress the progression of diabetes. Serum insulin level is elevated with lupeol treatment. Concomitantly it causes reduction of glycated haemoglobin, serum glucose and nitric oxide. Thus lupeol works as a potential antidiabetic constituent (Gupta et al., 2012).

CONCLUSION

From the above information it is clear that S. cumini possesses potential antidiabetic compounds which are of utmost importance and is therefore imperative to further investigation. Out of four valuable constituents found, stigmasterol is one of the major one and has been isolated from many plants till date and evaluated for antidiabetic activities. Further studies should be carried out in order to explore the exact mechanism of these compounds to use Syzygium cumini (L.) Skeels. as a potential antidiabetic medicinal plant.

STATEMENT OF CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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