Phytomedicine: A novel alternative for treatment of gout

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Abstract

Gout is a disorder of purine metabolism in which final product that is uric acid gets precipitated in the form of monosodium urate crystal in joints and in the surrounding tissues. These crystals then lead to local immune-mediated inflammatory reaction which involves IL-β, one of the key proteins in the inflammatory cascade. Due to the loss of uricase during the course of evolution in human and higher primate, has made this condition much common. Gout has been a medical problem for centuries and has increased in prevalence across the world. Despite the availability of allopurinol and febuxostat as xanthine oxidase inhibitors for the treatment of gout, some people develop allergic reactions to these drugs. Therefore, focus on research has increased on phytomedicines that act as inhibitors of xanthine oxidase. Thus, uses of medicinal plants to treat gout and related diseases are gaining much importance.

Key words: Gout, uricase, IL-β, xanthine oxidase, phytomedicine

1. Introduction

1.1 Purine metabolism

In humans, uric acid is the final oxidation (breakdown) product of purine metabolism. Uric acid is formed primarily in the liver and excreted by the kidney into the urine. During purine metabolism, guanosine monophosphate (GMP) is split into the base guanine and ribose.

Guanine is deaminated to xanthine. Similarly, adenosine monophosphate (AMP) is deaminated by the enzyme AMP deaminase to inosine monophosphate (IMP) from which the ribose unit is removed by the enzyme xanthine oxidase to form hypoxanthine. Xanthine is oxidized by oxygen and xanthine oxidase with the production of hydrogen peroxide and uric acid (Figure 1).

During early primate evolution, a nonsense codon inserted into its uricase gene and uricase, thus, produced as a truncated, 10 amino acids long, inactive protein fragment in humans and apes (Bomalaski et al., 2002). The biological reason for the loss of uricase activity in humans and certain primates is still not clear. According to one view, this loss has had a distinctly beneficial effect. It has been proved that uric acid is a powerful antioxidant and a scavenger of free radicals. Therefore, these properties of uric acid have contributed to a decreased cancer rate and a lengthened hominoid life span (Friedman et al., 1985).

Figure 1: Route of purine degradation in animals (Hayashi et al., 2000)

Due to absence of uricase enzyme in humans, the plasma concentration of uric acid is high (Colloc’h et al., 2006) and an abnormal rise of uric acid can lead to the development of disease known as gout (Figure 2) and hyperuricemia.
Hyperuricemia has been found to be associated with urate precipitation with the formation of crystals that are involved in gout, tumor lysis syndrome (TLS), chronic kidney disease (CKD), and various cardiovascular diseases (Edwards, 2008; Feig, 2009; Richette and Bardin, 2010). Gout remains among the most common of all inflammatory arthritis, due to lifestyle and dietary factors, such as heavy consumption of beer and liquor as well as diets rich in meats and seafood as important gout risk factors (Saag and Mikul, 2005). The normal serum urate level of uric acid is between 2.18-7.7 mg/dL. In gout, deposition of uric acid crystal takes place in and around the joints (Figure 3). The treatment of hyperuricemia has been performed with drugs that decrease amount of uric acid synthesis by inhibiting xanthine oxidase enzyme.

1.2 Conventional treatment for gout

Therapy for hyperuricemia-associated diseases has been treated by agents that keep plasma urate concentrations from precipitating with the additional benefit of removing existing urate crystals. Tumor lysis syndrome (TLS) is characterized by the acute onset of hyperuricemia during cancer chemotherapy (Davidson et al, 2004; Edwards, 2009; Mughal et al., 2010). Gout, with a prevalence of about 1%, is characterized by the sustained elevation of plasma urate levels (Chohan and Becker, 2009; Richette and Bardin, 2010). Chronic kidney disease is usually associated with hyperuricemia and has a prevalence of about 5% (Kang and Nakagawa, 2005; Sestigiani et al., 2008; Feig, 2009). Currently, available anti-hyperuricemia drugs act on xanthine oxidase, the urate transporter or urate itself. Allopurinol and febuxostat are antihyperuricemia drugs that competitively inhibit xanthine oxidase. Allopurinol has been used as first-line drug for treating gout and TLS, but refractory gout occurs when patients suffer from hypersensitivity or non-responsiveness to allopurinol, intolerance to allopurinol toxicity, or drug-drug interactions with allopurinol (Edwards, 2009; Keenan and Pillinger, 2009; and Mughal et al., 2010).Febuxostat has been reported to be tolerated by most of patients (Terkeltaub, 2010), but its efficacy in treating refractory gout has not been proven. Probenecid is antihyperuricemia drug acting on the renal urate transporter, but it has not been widely used due to toxic effect on kidney and liver (Chohan and Becker, 2009; Richette and Bardin, 2010).

However, for quick reduction of serum uric acid level, uricase enzyme has prominently been used. Uricase (urate oxidase, EC 1.7.3.3.) degrades the poorly soluble uric acid (~11 mg/100 ml H2O), into the more soluble product allantoin (~147 mg/100 ml H2O). Urate oxidase has been found in mammalian, plants, and microbial cells. The enzyme may be obtained from several microorganisms of the genus Micrococcus, Brevibacterium, Streptomyces, Candida, Bacillus, Pseudomonas, Arthrobacter, and Aspergillus. We have isolated a novel Alcaligenes sp. for the production of uricase for the treatment of hyperuricemia (Figure 4). Uricase from Alcaligenes sp. was purified to homogeneity by ammonium sulphate precipitation and DEAE column chromatography. The subunit molecular mass of purified uricase was found to be 30 kDa (Figure 5).
The kinetic parameters $K_m$ and $V_{max}$ of the purified uricase from *Alcaligenes* sp. were determined by measuring the uricase activity at different uric acid concentrations (0.01-0.07% w/v). Reaction velocity at varying substrate concentration was determined and a graph was plotted between $1/ [S]$ vs. $1/ [V]$. The $K_m$ and $V_{max}$ values, were calculated from Lineweaver-Burk plot. The $K_m$ and $V_{max}$ of uricase from *Alcaligenes* sp. was found to be approximately 4.27 mM and 90.9 $\mu$mol/min/mg, respectively (Figure 6).

Gout prevalence has been increased in direct association with age and the increased longevity of populations in industrialized nations through the disorders associated with age-related diseases such as metabolic syndrome and hypertension (Ar’ev et al., 2012). Gout can also cause ocular surface abnormalities, such as tophi deposition, subconjunctival transparent vesicles and vascular changes. These features have provided important clinical significance in early detection of the gout and prevention of eye injury (Lin et al., 2013).

The increase in the prevalence of gout worldwide indicates that there is an urgent need for improved efforts to treat the gout. Currently available therapies for gout (Figure 7) have serious adverse side effect on human body. Since, nature contains rich source of medicinal plants and some of them have been reported to inhibit xanthine oxidase (Azmi et al., 2012). These medicinal properties of plants can be used as new natural sources of gout medication and a substitute for synthetic xanthine oxidase inhibitors.

### 1.3 Medicinal plant as alternative to treat gout

Plants contain high amount of antioxidant and biologically active compounds and, thus, acts as target for the searching the new drugs (Gautam et al., 2012; Thakur and Azmi, 2013). The treatment of gout involves the use of therapeutic agents such as xanthine oxidase inhibitors (Kong et al., 2001; Unno et al., 2004). These inhibitor act by blocking the biosynthesis of uric acid from xanthine during purine metabolism in the body (Unno et al., 2004). The concentration of uric acid can be regulated by either increasing the excretion of uric acid or reducing the uric acid production to reduce the risk of gout (Umamaheswari et al., 2007).
Allopurinol is one of the synthetic xanthine oxidase inhibitor which has been widely used in the therapeutic and clinical management of gout and conditions associated with hyperuricemia as well as related inflammatory diseases (Fields et al., 1996; Pacher et al., 2006). However, drawback of using allopurinol is that it generates superoxide (Berry and Hare, 2004) and causes allergical reactions in body (Wallach, 1998; Kong et al., 2000).

Thus, the use of herbal plants to treat diseases is gaining new interest because of their low allergenicity (Unno et al., 2004). Plants are important sources of medicines and in United States, about 25% of pharmaceutical prescriptions contain at least one plant-based ingredient. Based on the traditional knowledge obtained from various sources, roughly 121 pharmaceutical products were formulated during the last century. Phytomedicine obtained from herbal sources are in great demand in the developed world as their ability to cure many diseases. These plant based drugs provide outstanding contribution to modern therapeutics (Pandey et al., 2011). Some of herbal plants and their phytochemicals are worth to be explored as potential xanthine oxidase inhibitor as they are already used as food or food supplements for many years and found safe for human bodies (Abd Aziz et al., 2011). The herbs or natural medicines having the potential for inhibiting xanthine oxidase are summarized in Table 1 (Kong et al., 2000; Umamaheshwari et al., 2007).

Polyphenols (Costantino et al., 1992), flavonoids (Chang et al., 1993; Selloum et al., 2001), coumarins (Chang and Chiang, 1995), ellagic acid, valoneic acid dilactone (Unno et al., 2004) have been reported to be potent plant-based xanthine oxidase inhibitor.

Table 1: List of herbs that inhibit xanthine oxidase

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Local name</th>
<th>Percent of xanthine oxidase inhibition (50-100μg/mL)</th>
<th>IC₅₀ (μg/mL)</th>
<th>Part of plant used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelica dahurica</td>
<td>Angelica root</td>
<td>40-90%</td>
<td>NR</td>
<td>Root</td>
</tr>
<tr>
<td>Angelica sinensis</td>
<td>Angelica root head</td>
<td>18-93%</td>
<td>40</td>
<td>Root</td>
</tr>
<tr>
<td>Artemisia anomola</td>
<td>Sweet gum fruit</td>
<td>48-89%</td>
<td>33-66</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Astragalus membranaceus</td>
<td>Astragalus root</td>
<td>12-64%</td>
<td>85</td>
<td>Root</td>
</tr>
<tr>
<td>Carthamus tinctorius</td>
<td>Safflower</td>
<td>23-81%</td>
<td>64</td>
<td>Fruit</td>
</tr>
<tr>
<td>Chrysanthemum indicum</td>
<td>Chrysanthemum flower</td>
<td>34-95%</td>
<td>24-91</td>
<td>Flower</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>Orange</td>
<td>27-51%</td>
<td>98</td>
<td>Fruit shell</td>
</tr>
<tr>
<td>Cledodendron trichotomum</td>
<td>Harlequin glory bower</td>
<td>12-86%</td>
<td>65</td>
<td>Leaf</td>
</tr>
<tr>
<td>Coccinia grandis</td>
<td>Tondi</td>
<td>40-77%</td>
<td>21-32</td>
<td>Leaf</td>
</tr>
<tr>
<td>Cuscuta chinensis</td>
<td>Cuscuta seed</td>
<td>23-55%</td>
<td>53</td>
<td>Seed</td>
</tr>
<tr>
<td>Datura metal</td>
<td>Angel or Devil’s trumpet</td>
<td>9.4-62%</td>
<td>77</td>
<td>Leaf</td>
</tr>
<tr>
<td>Erythrina indica</td>
<td>Tiger claw</td>
<td>17-79%</td>
<td>70</td>
<td>Bark</td>
</tr>
<tr>
<td>Fraxinus rhynchophylla</td>
<td>Flaxinus, Ash</td>
<td>48-96%</td>
<td>28-53</td>
<td>Bark</td>
</tr>
<tr>
<td>Glechoma longitube</td>
<td>Glechoma</td>
<td>16-91%</td>
<td>48</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Glycyrrhiza uralensis</td>
<td>Lico rice root</td>
<td>24-49%</td>
<td>NR</td>
<td>Root</td>
</tr>
<tr>
<td>Kochia scoparia</td>
<td>Kochia fruit</td>
<td>24-51%</td>
<td>116</td>
<td>Seed</td>
</tr>
<tr>
<td>Ligusticum brachylobum</td>
<td>Ligusticum</td>
<td>17-94%</td>
<td>34</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Lycopodium clavatum</td>
<td>Lycopodium tuber</td>
<td>13-58%</td>
<td>94</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Lycopus europaeus</td>
<td>Gypsywort, Bagleweed</td>
<td>39-93%</td>
<td>26-79</td>
<td>Leaf</td>
</tr>
<tr>
<td>Morus alba</td>
<td>White mulberry</td>
<td>18-93%</td>
<td>57</td>
<td>Twig</td>
</tr>
<tr>
<td>Piper kadsura</td>
<td>Piper</td>
<td>11-94%</td>
<td>28</td>
<td>Stem</td>
</tr>
<tr>
<td>Plantago asiaticum</td>
<td>Plantago</td>
<td>26-56%</td>
<td>98-103</td>
<td>Seed, Ariel</td>
</tr>
<tr>
<td>Polygonum cuspidatum</td>
<td>Knotweed</td>
<td>12-94%</td>
<td>38</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Prunella vulgaris</td>
<td>Selfheal spike</td>
<td>27-63%</td>
<td>86</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Rheum palmatum</td>
<td>Rhabarb rhizome</td>
<td>17-57%</td>
<td>101</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Salvia miltiorrhiza</td>
<td>Salvia root</td>
<td>27-56%</td>
<td>96</td>
<td>Root</td>
</tr>
<tr>
<td>Scutellaria barbata</td>
<td>Scutellaria, Barbal skull cap</td>
<td>31-87%</td>
<td>62</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Smilax china</td>
<td>Prickly ivy’s, catbriers</td>
<td>18-89%</td>
<td>62</td>
<td>Rhizome</td>
</tr>
<tr>
<td>Strychnos nox-vomica</td>
<td>Strychnine tree</td>
<td>38.7-82%</td>
<td>6.8-32</td>
<td>Leaf</td>
</tr>
<tr>
<td>Vitex negundo</td>
<td>Chaste tree</td>
<td>6.2-66%</td>
<td>76-88</td>
<td>Leaf</td>
</tr>
</tbody>
</table>

Source: Modified from Kong et al., 2000; Umamaheshwari et al., 2007

* IC₅₀ represents different form of extract (Me-OH; Me-OH-H₂O; H₂O) NR=Not reported
The type of herbal extract used such as methanol (Me-OH), Me-OH-H2O, or H2O also have a significant influence on the degree of inhibition of herbal product, has on xanthine oxidase activity. In most of the cases, the herbs prepared as a Me-OH extract has been found to be a greater inhibition of xanthine oxidase as compared to other types of extracts of the same herb (Umamaheshwari et al., 2007). Similar effect has been found to be with other herbs or natural medicines that are known to inhibit xanthine oxidase activity from other regions of the world (Kong et al., 2000).

However, many studies have reported that herbal medicine has beneficial effect on patients with gout. The herbs which have been used in treatment were chosen either their ability to eliminate uric acid (Atriplex graveolens, Urtica spp, Taraxacum officinale) or anti-inflammatory (Harpagophytum procumbens, Filipendula ulmaria, Salix spp., Betula spp, Curcuma longa and Guaiacon spp. as reported by Nadia and Barbara, 2013).

2. Indian medicinal plant as antigout agent

2.1 Gloriosa superba Linn.

Gloriosa superba Linn. is a well known ethnomedical plant which has also been mentioned in Ayurveda (Figure 8a). Phytochemical studies has been reported on G. superba, shown the presence of colchicin, b-sitosterol, long chain fatty acids, b and g-lumic colchicines, 2-hydroxy-6-methoxy benzoic, lurtern, N-formyl-de acetyl colchicines and new colchicine glycoside, 3-O-demethylcolchicine-3-O-alpha-D-glucopyranoside. Gloriosa superba reproduce by corm and seeds but has very low germination capability which restricts its multiplication. Therefore, biotechnological technique can be very useful in order to increase the efficiency of germination of this important plant. Micropropagation of Gloriosa superba can help in meeting the increasing demands for colchicine. Thus, Gloriosa superba can acts as potential source of colchicine in India due to availability from both wild and cultivated sources (Hemant et al., 2011). FDA has also approved the use of colchicine is to treat gout. It is one of the active ingredients of antigout tablets marketed by Merck and Co. (Ling and Bochu, 2014).

2.2 Erythrina indica Lam.

Erythrina indica Lam. belongs to the family Leguminosae (Figure 8b). This is an important medicinal plant and found in wild deciduous forests throughout India and in Andaman and Nicobar Islands. Bark of E. indica has been used medically as febrifuge, antibilious and also used to treat dysentery. Bark powder has been traditionally used for rheumatism, itching, fever, asthma and leprosy (Anonymous, 1992). The study has shown that the root bark of E. indica has been used for the treatment of trachoma, elephantiasis, and microbial infections (Nkengfa et al., 2001). Different kinds of pholic compounds including flavonoids derivatives and various biologically active metabolites have been isolated from the bark of this plant (Azebaze et al., 2000; Nkengfa et al., 2001). There has been numerous reports on the antioxidant and free radical scavenging potential of leaves and barks of E. indica (Sakat and Juvekar, 2010) by measuring the increase in absorbance at 295nm associated with uric acid formation. The methanol extract of stem bark of E. indica contains higher level of total phenolic content (412.8 mg GAE/g extract) having xanthine oxidase inhibition activity (Kandhasamy et al., 2012). Thus, it can be used for treating gout.

2.3 Citrus aurantium Linn, Citrus limetta W. and A. and Citrus limon (Linn) Burm.

Many Indian medicinal plants have been used for the prevention and treatment of gout and related inflammatory disorders. Citrus limetta, Citrus aurantium and Citrus limon (Figure 8c, d, e) belonging to the family Rutaceae, which is traditionally used by the local people and tribes in India for the treatment of gout, liver disorders, stomachic, brain troubles, etc. (Figure 8c). These medicinal plants possess phytochemical constituents like flavonoids, and a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity. The in vitro xanthine oxidase inhibitory activity of the extract of leaves, fruits and peel of Citrus aurantium, Citrus limettas, and Citrus limon has been determined. These leaf and peel extracts of C. aurantium, C. limetta and C. limon and the fruit extract of C. limetta) has shown xanthine oxidase activity at a concentration of 100%/g/ml showing an inhibition greater than 50% (Muthiah, 2012). Since, alternatives with an increased therapeutic activity and less side effects are desired. Thus, the use of the medicinal plants can act as the alternative to chemical drugs such as allopurinol and can be used for treatment of gout.

2.4 Tephrosia purpurea (Linn.) Pers. Tephrosia purpurea (Linn.) Pers. (Fabaceae) is a pantropical, polymorphic, branched, suberect, perennial herb popularly known as Sarapunkha in Sanskrit (Figure 8f), Purple Tephrosia in English and Unali in Marathi (Kirtikar and Basu, 1975). The plant is used in folk medicine as an antidiabetic, antipyretic, anticancer and antitumor agent in addition to its usefulness in treatment of diseases related to oxidative stress and free radicals activity (Pavana et al., 2008). Reactive Oxygen Species (ROS) play important role in the initiation and progression of various diseases such as artherosclerosis, cardiovascular diseases, aging, respiratory diseases, cancer and gout (Estebauer et al., 1991; Ames and Shigenaga, 1992). Root extract of T. purpurea has been evaluated as an antioxidant, anti-inflammatory and potent inhibitor of xanthine oxidase which is mainly involved in formation of uric acid, leading to free radical induced damage and gout. The phytochemical analysis revealed presence of significant amount of polyphenols and flavonoids. T. purpurea root extract possesses prominent medicinal properties and can be exploited as natural drug to treat the diseases associated with free radical formation, oxidative stress and xanthine oxidase activity (Nile and Khobragade, 2011).
known as ‘Kureel’ or Kareel in Hindi (Ozacus, 1999). Plant has its wider utility in traditional folk medicine and has been used as ailments to relieve variety of pains or aches such as toothache, cough and asthma heal (Ravi, 2011). Phytochemical studies of C. decidua have shown presence of many beneficial compounds, which have shown analgesic, laxative, antihelminthic, antiparasitic and antiprotozoan activity. Root bark powder has been traditionally used to cure rheumatism, dropsy, ulcer, gout, fever, cough, asthma, boils, piles and inflammation (Chakravarty and Venkarasubramaniam, 1932). This plant contains few important secondary metabolites such as quercetin which act as melanogenesis stimulator and also increase tyrosinase protein expression (Lam and Ng, 2009). Capparis sp. seeds contain lectin that exhibit potent anti HIV-1 reverse transcriptase inhibition activity and also inhibits proliferation of hepatoma HepG2 and breast cancer MCF-7 cells (Leucha et al., 2009). It has also been reported for having antirheumatic, antidiabetic, antiarthritis and antigout effect (Chakravarty and Venkarasubramaniam, 1932). C. decidua has been reported to contain generous quantities of alkaloids, fatty acids, terpenes, vitamins, fibre and oils that show greater medicinal and nutritive value (Sharaf et al., 2000). It also contains saccharides, glycosides, flavonoids, volatile oils, sterols and steroids, which showed multiple pharmacological effects such as antigout, odynolysis, antifungus, hepatoprotective effect, hypoglycemic activity, antioxidation, antihyperlipemia, anticoagulated blood, smooth muscle stimulation and antistress reaction (Calis et al., 2002).
3. Conclusion

Plant contain mixture of complex compounds having biological properties such as antioxidant, detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, and modulation of hormone metabolism. The bioactive compounds that have been derived from plants include flavonoids, terpenoids, phenolic acids, and other categories of phytochemicals based on their structure, has been reported for their antigout and anti-hyperuricemic effect. Since, the prevalence of gout has been increased all around the world at steady rate and conventional therapies for gout treatment have been restricted due to their failure to provide long term immunity, high cost and their side effect on human body. Thus, the screening of various herbal plants extract for antigout effect and lesser side effects, can lead to development of novel drug therapy for gout treatment.

Acknowledgement

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Conflict of interest

We declare that we have no conflict of interest.

References


2.6 Polyalthia longifolia (Sonn.) Thwaites

This plant belongs to Annonaceae family, is a lofty evergreen tree, native to India and commonly planted due to its effectiveness in alleviating noise pollution (Figure 8h). The plant has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helmintiasis (Kritikar and Basu., 1995). The plant was reported to possess antibacterial, cytotoxic, antinulear and antifungal activities (Jain et al., 2006; Nair and Chanda 2006a; Nair and Chanda 2006b; Malairajan et al., 2008). The study has reported the in vitro xanthine oxidase inhibitory activity of the extracts of the leaves of Polyalthia longifolia. The study has shown that the ethanol and chloroform extracts of leaves of Polyalthia longifolia exhibit xanthine oxidase inhibitory activity (Andichettiar and Tapan, 2012). In traditional medicine, the plant has been used in various disorders including diabetes. Xanthine oxidase is reported to be involved in the chronic complications, associated with diabetes mellitus. Further, investigations on the active phytoconstituents in the plant and in vivo studies are necessary to ascertain the mechanism and usefulness of this plant in preventing gout complications, associated with chronic diabetes.


