Comparative study of *Acacia nilotica* and *Acacia sinuata* for diuretic activity


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**ABSTRACT**

Diuretics increase the rate of urine outflow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis. Some naturally used diuretic plants are Abrus precatorius, Centella asiatica, Allium sepa, Allium sativum, Aloe barbadensis, Cinnamomum verum etc. Traditionally *Acacia nilotica* is used as anti-cancer, astringent, antioxidant, antispasmodial, diuretic and anti-diarrhoeal. *Acacia sinuata* is used in the treatment of skin disease, burning sensation, constipation, calculi, hemorrhoids, vitiligo, eczema and malaria fever. The present study was undertaken to investigate diuretic effect of ethanolic and methanolic extracts of stem bark of *Acacia nilotica* and pods of *Acacia sinuata* in wistar rats. The extracts were administered orally at a dose of 300mg/kg. Furosemide (20mg/kg) was used as reference standard and normal saline (25 ml/kg) was used as control. Total urine volume and concentration of Na$^+$, K$^+$ and Cl$^-$ excreted in urine were estimated. It was concluded that both ethanolic and methanolic extracts of *Acacia nilotica* and *Acacia sinuata* showed significant diuretic activity. *Acacia nilotica* showed more potent diuretic activity than *Acacia sinuata* and of these, ethanolic extract showed more significant activity than methanolic extract.

**Key words:** *Acacia nilotica*, *Acacia sinuata*, Diuretic, Furosemide, Urine volume.

**INTRODUCTION**

Since the time immemorial, our traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully[1]. India has a rich heritage of using medicinal plants and hosting several thousands of medicinally valuable plants belonging to hundreds of families. One cannot assure that all of these plants possess a long recorded history, although they have been reported to contain medicinally valuable phyto-pharmaceuticals and subjected to formulate, ayurvedic, unani, siddha, Chinese system of medicine[2]. Diuresis is an increase in the production of urine by the kidneys, which typically results in a corresponding increase in urine expelled by the body, diuresis without an
accompanying increase of urination can cause severe medical problems [3]. Drugs that induce diuresis are known as diuretics [4]. Diuretics relieve pulmonary congestion and peripheral edema. This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure. Thus, diuretics play an important role in hypertensive patients [5], in situations of fluid overload like acute and chronic renal failure, hypercalciuria, cirrhosis of liver and also as an antihypertensive agent. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na+ reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH [6]. A number of diuretics like mannitol, thiazides, furosemide and ethacrynic acid are used in practice [7].

Furosemide, a potent diuretic agent that induces a powerful diuresis, followed by the loss of sodium, potassium, and chloride into urine, by acting on thick ascending limb of the loop of henle [8].

Acacia nilotica is a medium-sized, thorny, evergreen tree with spreading crown; thorns straight, 2-5 cm long, white. Leaves 2-pinnate, pinnae 6-12, leaflets small, 20-40. Flowers yellow, in globose axillary heads. Fruits moniliform, compressed, constricted at the sutures between the seeds [9].

Acacia sinuata is a perennial, woody, large climbing shrub grows on big trees. Leaves are bipinnate, leaflets small, sessile, flowers small heads, fruits thin pods with 6-10 seeds per pod.

The stem bark of Acacia nilotica and pods of Acacia sinuata were well-known for its diuretic properties. So far no scientific evidence was observed for the claimed activity of this product. In this regard we have aimed to identify the diuretic effect of both ethanolic and methanolic extracts of Acacia nilotica and Acacia sinuata.

MATERIALS AND METHODS

Plant materials
The stem bark of Acacia nilotica and pods of Acacia sinuata belonging to family Mimosaceae were collected from local area of Anantapur district (India) and was identified and authenticated by Dr. J. Ravindra Reddy, M. Pharm, Ph.D, Department of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur and voucher specimen (riper-10/11) is preserved in Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur.

Drugs and chemicals
Furosemide (Aventis Pharma Ltd), Ethanol (Merck Pharmaceuticals Pvt Ltd), Methanol (Merck Pharmaceuticals Pvt Ltd).

Animals
Wistar rats of 200-250g were used to carry out the diuretic activity. The animals had free access to standard commercial diet and water ad libitum and were housed in cages under standard laboratory conditions i.e., 12:12 hour light/dark cycle at 25 ± 2°C. The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/011/2011) has approved the experimental protocol at Post Graduate Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, India.
Preparation of ethanolic extract of *Acacia nilotica*

The stem bark of *Acacia nilotica* was extracted by soxhlet extraction by using 400ml of ethanol. In the extraction procedure a total amount of 50 gm powdered material were used. The extract was distilled and then it was concentrated. The percentage yield of ethanolic extract of *Acacia nilotica* was found to be 13% (Table-1).

Preparation of methanolic extract of *Acacia nilotica*

The stem bark of *Acacia nilotica* was extracted by maceration process by using 300ml of methanol. In the maceration procedure a total amount of 100 gm powdered material were macerated for three days. The extract was filtered and then it was concentrated. Then it was dried by rotary evaporator. The percentage yield of methanolic extract of *Acacia nilotica* was found to be 10% (Table-1).

Preparation of ethanolic extract of *Acacia sinuata*

The pods of *Acacia sinuata* was extracted by soxhlet extraction by using 400ml of ethanol. In the extraction procedure a total amount of 50 gm powdered pods were used. The extract was distilled and then it was concentrated. The percentage yield of ethanolic extract of *Acacia sinuata* was found to be 12% (Table-1).

Preparation of methanolic extract of *Acacia sinuata*

The pods of *Acacia sinuata* was extracted by maceration process by using 300ml of methanol. In the maceration procedure a total amount of 100 gm powdered pods were macerated for three days. The extract was filtered and then it was concentrated. Then it was dried by rotary evaporator. The percentage yield of methanolic extract of *Acacia sinuata* was found to be 14% (Table-1).

Phytochemical analysis

Ethanolic and Methanolic extracts of stem bark of *Acacia nilotica* and pods of *Acacia sinuata* were subjected to phytochemical screening [10].

Diuretic activity

Healthy Wistar rats of either sex weighing around 200 -250g taken. The rats were divided into six groups of six animals each. Ethanolic and methanolic extracts of both stem bark of *Acacia nilotica* and pods of *Acacia sinuata* were evaluated for diuretic activity. Furosemide (20mg/kg) was used as reference standard. Before the experiment, the rats were fasted for 18 hours with free access to water. On the day of experiment, the animals of group 1 were administered saline (25ml/kg, p.o.) [11] and this group served as control. Group 2 rats were administered with standard drug furosemide (20mg/ kg, p.o.). Group 3 and Group 4 rats received ethanolic and methanolic extracts (300mg/kg, p.o.) of *Acacia nilotica* respectively. Similarly the Group 5 and Group 6 rats received ethanolic and methanolic extracts (300mg/kg, p.o.) of *Acacia sinuata* respectively. Immediately after the treatment the rats were placed in metabolic cages and the urine samples were collected for 5h, measured using a standard measuring cylinder. The amount of urine (in ml) collected for 5 h was compared and tabulated [12]. The parameters measured were total urine volume, urine concentration of Na⁺, K⁺ and Cl⁻. Concentration of Na⁺ and K⁺ were determined using flame photometer while Cl⁻ concentration was estimated titrimetrically using 0.02N AgNO₃ with 5% potassium chromate as an indicator. Appearance of brick red precipitate was taken as the end point [13].
Statistical Analysis
The results were expressed as mean ± S.E.M. The differences were compared using One Way Analysis Of Variance (ANOVA) and subsequently followed by Bonferroni’s test.

RESULTS

Table 1: %Yield, texture and colour of ethanolic and methanolic extracts of Acacia nilotica and Acacia sinuate

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Type of Extract</th>
<th>% Yield</th>
<th>Texture</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem bark of Acacia nilotica</td>
<td>Ethanolic extract</td>
<td>13</td>
<td>Gummy</td>
<td>Reddish brown</td>
</tr>
<tr>
<td></td>
<td>Methanolic extract</td>
<td>10</td>
<td>Gummy</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>Pods of Acacia sinuata</td>
<td>Ethanolic extract</td>
<td>12</td>
<td>Gummy</td>
<td>Reddish brown</td>
</tr>
<tr>
<td></td>
<td>Methanolic extract</td>
<td>14</td>
<td>Gummy</td>
<td>Reddish brown</td>
</tr>
</tbody>
</table>

Phytochemical analysis:
Both ethanolic and methanolic extracts of Acacia nilotica and Acacia sinuata revealed the presence of alkaloids, glycosides, saponins, tannins, aminoacids, steroids and terpenoids (Table-2).

Table 2: Phytochemical analysis of ethanolic and methanolic extracts of Acacia nilotica and Acacia sinuata

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Acacia nilotica</th>
<th>Acacia sinuata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanolic extract</td>
<td>Methanolic extract</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aminoacids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present

Effect of extracts on urine output and electrolyte excretion in rats:
The urine volume and concentrations of Na⁺, K⁺ and Cl⁻ in ethanolic and methanolic extracts of Acacia nilotica (300mg/kg) were 9.4ml, 127.8mEq/L, 87.43mEq/L, 155.8 mEq/L and 6.6ml, 113.1mEq/L, 69.17mEq/L, 120.3 mEq/L respectively (Table-3).

Table 3: Effect of ethanolic and methanolic extracts of Acacia nilotica on urine output and electrolyte excretion in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose &amp; Route</th>
<th>Urine volume (ml)</th>
<th>Electrolyte concentration in urine (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>25 ml/kg p.o.</td>
<td>4.03 ± 0.12</td>
<td>Na⁺ 90.0 ± 0.61, K⁺ 51.73 ± 2.80, Cl⁻ 97.3 ± 1.04</td>
</tr>
<tr>
<td>Furosemide</td>
<td>20 mg/kg p.o.</td>
<td>9.267 ± 0.08</td>
<td>Na⁺ 136.8 ± 1.55, K⁺ 94.17 ± 1.18, Cl⁻ 163.7 ± 1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.43 ± 0.12</td>
<td>127.8 ± 1.28, 87.43 ± 1.09, 155.8 ± 2.02</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>300 mg/kg p.o.</td>
<td>6.60 ± 0.11</td>
<td>113.1 ± 1.79, 69.17 ± 0.69, 120.3 ± 2.28 ***</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>300 mg/kg p.o.</td>
<td>6.60 ± 0.11</td>
<td>113.1 ± 1.79, 69.17 ± 0.69, 120.3 ± 2.28 ***</td>
</tr>
</tbody>
</table>

**** p <0.001 , ***p<0.01 when compared to control.

The urine volume and concentrations of Na⁺, K⁺ and Cl⁻ in ethanolic and methanolic extracts of Acacia sinuata (300mg/kg) were 8.9ml, 116.5mEq/L, 73.77mEq/L, 136.9 mEq/L and 5.5ml, 104.5mEq/L, 64.67mEq/L and 114.5 mEq/L respectively (Table-4).
Table 4: Effect of ethanolic and methanolic extracts of *Acacia sinuata* on urine output and electrolyte excretion in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose&amp; Route</th>
<th>Urine volume (ml)</th>
<th>Electrolyte concentration in urine (mEq/L)</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline)</td>
<td>25 ml/kg p.o.</td>
<td>4.03 ± 0.12</td>
<td>90.0 ± 0.61</td>
<td>51.73 ± 2.80</td>
<td>97.3 ± 1.04</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>20 mg/kg p.o.</td>
<td>9.267 ± 0.08****</td>
<td>136.8 ± 1.55****</td>
<td>94.17 ± 1.18****</td>
<td>163.7 ± 1.30****</td>
<td></td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>300 mg/kg p.o.</td>
<td>8.90 ± 0.05****</td>
<td>116.5 ± 0.69****</td>
<td>73.77 ± 0.97****</td>
<td>136.9 ± 1.59****</td>
<td></td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>300 mg/kg p.o.</td>
<td>5.56 ± 0.08****</td>
<td>104.5 ± 0.62****</td>
<td>64.67 ± 1.31</td>
<td>114.5 ± 1.90****</td>
<td></td>
</tr>
</tbody>
</table>

* **** p <0.001, *** p<0.01, ** p<0.05 when compared to control.

**DISCUSSION**

Diuretics relieve pulmonary congestion and peripheral oedema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure[14].

The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles [15]. The regulation of sodium, potassium balance is also intimately related to renal control of acid-base balance. The chemical constituents may be responsible for diuretic activity include alkaloids, glycosides, saponins, tannins, aminoacids, steroids and terpenoids [16]. In the present study we can demonstrate that ethanolic and methanolic extracts may produce diuretic effect by increasing the excretion of sodium, potassium and chloride. These features suggest that the plant extract is acting in a similar way as furosemide, which increases urinary output and urinary excretion of sodium by inhibiting Na+/K+/Cl- transporter system in the thick ascending loop of Henley [17].

The possible mechanism of action of phytochemical constituents that are responsible for diuretic action may be Saponins due to local irritation of kidney epithelium [18]. Alkaloids due to vasodilatory action on renal blood vessels [19]. Glycosides due to increased blood flow to the kidneys from enhanced cardiac contractility [20].

**CONCLUSION**

Ethanolic extract of *Acacia nilotica* and *Acacia sinuata* showed significant increase in urine volume and concentrations of Na⁺, K⁺, Cl⁻ when compared to methanolic extract of *Acacia nilotica* and *Acacia sinuata*. Of these both, *Acacia nilotica* showed more potent diuretic activity than *Acacia sinuata*.

**Acknowledgements**

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