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## Preliminary phytochemical screening and GC-MS analysis of leaf extract of *Acacia catechu* (L.f.) Willd

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### Abstract

*Acacia catechu* is a deciduous tree and reported to possess therapeutic properties. In the present study fifty percent hydromethanolic extract of leaves of *Acacia catechu* (ACE) was prepared and analyzed for presence of various phytoconstituents in ACE employing different biochemical qualitative tests and GC-MS analysis. It was further explored for its antioxidative potential through hydrogen peroxide scavenging assay. Biochemical tests confirmed presence of various phytochemicals viz., resins, tannins, Saponins, flavonoids, alkaloids, glycosides, etc. GC-MS analysis showed presence of four compounds when compared with NIST and WELLY library. *Acacia catechu* (A. *catechu*) showed presence of Caprylic acid methyl ester (19.77%), Lauric acid methyl ester (27.42%), 2-Ethyl-3-methyl-1-butene (42.09%) and Myristic acid methyl ester (10.72%), etc. The present study indicated anti-oxidative property of ACE. The percent H<sub>2</sub>O<sub>2</sub> scavenging activity of ACE was found to be 33.40%. Thus it could be inferred that ACE displayed presence of various phytoconstituents which could be responsible for its antioxidative potential.

**Keywords:** *Acacia catechu*, GC-MS analysis, Phytochemical Screening; hydrogen peroxide scavenging assay.

### 1. Introduction

*Acacia catechu* (Kath) is a genus of shrubs and trees belonging to the sub-family Mimosoideae and of the family Fabaceae, first described in Africa. *Acacia catechu* has many nutritional properties and medicinal uses. It is a medium-sized, thorny deciduous tree grows up to 13 meters in height. Leaves are bi-pinnately compound, leaflets 30-50 paired, main rachis pubescent, with large conspicuous gland near the middle of the rachis. Flowers are pale yellow, sessile, found in axillary spikes. Fruits show flat brown pods, with triangular beak at the apex, shiny, narrowed at base. There are 3-10 seeds per pod. The gummy extract of the wood is called katha or cutch [1, 2].

Major phytoconstituents present in *Acacia catechu* are catechin, epicatechin, gallate, protocatechuic acid, tannins, alkaloids quercetin and kaempferol. Porifera sterol glucosides and afzelechin gum are also present in minor quantity [3]. The chief constituents of the heartwood and leaves of *Acacia catechu* are catechin and catechu tannic acid [4]. The extract prepared from the hard wood of *Acacia catechu*, has been used for treating fever, diarrhoea, leucorrhoea, piles and erysipelas [5]. The leaves contain 13.03-18.72% crude protein, 46.69-50.96% N free extract and 0.14-0.17% phosphorus. Total digestible nutrients are 46.33 kg of dry material and thus used as fodder for sheep, goats and cattle [6]. The juice of its fresh bark has been used in treatment of haemoptysis and gonorrhoea. Catechuic acid found in cavities of the wood of the *Acacia catechu* tree (Leguminosae) was valued for facilitating expectoration in chest infection [7]. Keeping in view the mentioned facts, study was planned to explore phytochemicals in fifty percent hydro-methanolic leaf extract of *Acacia catechu* (ACE) through qualitative biochemical and GC-MS analyses. The antioxidative potential was assessed through hydrogen peroxide scavenging assay.

### 2. Materials and Methods

#### 2.1 Plant material

The authentic plant material i.e., leaves of *Acacia catechu* were obtained from Agroforestry Research Centre, G.B.P.U.A. &T., Pantnagar, Uttarakhand, India.

#### 2.2 Preparation of Extract of *Acacia catechu* (ACE)

Leaves were washed properly, shed dried and ground into a fine powder and stored in sterile containers in a cool dry place till further use. Extraction of the plant material was carried out

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by using solvents with different polarities. Hydromethanolic extract was prepared as described by Ukwuani *et al.* [8]. In brief 50 gm of the powder was allowed to soak in 500ml 50% methanol (v/v) for 48 hours under continuous agitation in a shaking incubator. The mixture was first filtered through muslin cloth, then through Whatman filter paper No 1. The filtrate was then kept in the rotatory evaporator (45°C). Finally the extract (ACE) was obtained by drying the filtrate under hot circulating air at 40°C followed by lyophilization. The percent yield was calculated by dividing quantity of the plant extract obtained by 50 gm of dry powder. The prepared extract was kept at -20°C till further use.

### 2.3 Phytochemical Analyses of ACE

Qualitative phytochemical tests for the identification of carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenols and glycosides were carried out for ACE as per the methods described by Trease and Evans [9], Harborne [10] and Sazada *et al.* [11].

**2.3.1 Test for Proteins:** Few drops of nitric acid were added by the sides of the test tube very gently to 1 ml methanol extract. Formation of yellow colour indicated the presence of protein in the sample (Xanthoprotein test).

**2.3.2 Test for carbohydrates:** 1 ml each of Fehling A and Fehling B were added in diluted extract and heated for 30 minutes and observed for the formation of brick red colour.

**2.3.3 Test for Resins:** Five milliliter of distilled water was added to the methanol extract and observed for turbidity.

**2.3.4 Test for Tannins:** 5 ml of 45% ethanol was added to 2 g of the ground sample and boiled for 5 min. The mixture was cooled and filtered. Then 3 drops of lead sub acetate solution was added to 1 ml of the filtrate. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added to 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins.

**2.3.5 Test for Saponins:** 0.5 g of methanol extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a persistent froth. The frothing was mixed with 3 drops of Olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**2.3.6 Test for Flavonoids:** 0.5 g of the medicinal plant extract of was introduced into 10 ml of ethyl acetate and heated in boiling water for 1 min. The mixture was then filtered. 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of yellow colour in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids.

**2.3.7 Test for Alkaloids:** 5 gm of ground material was extracted with 10 ml Ammonical Chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M Sulphuric acid. Creamish white precipitate was observed for the presence of Alkaloids.

**2.3.8 Tests for Steroids:** 2 ml of acetic anhydride was added to 0.5 g of methanol extract and 2 ml of Sulphuric acid was added by the sides of the test tube and observed for the colour

change from violet or blue-green.

**2.3.9 Test for Phenols:** Methanol extract was taken in a test tube and mixed with distilled water and warmed. To this 2 ml of Ferric chloride solution was added and observed for the formation of green or blue colour.

**2.3.10 Test for Glycosides:** About 0.5 ml of methanol extract was taken in a test tube and added 1 ml glacial acetic acid containing traces of ferric chloride. To this solution 1 ml concentration Sulphuric acid was added and observe for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

### 2.4 Characterization of ACE by GC-MS analysis

The samples were analyzed at commercial facility of GC-MS analysis available at RITL, JNU, New Delhi, with the following parameters.

#### 2.4.1 Sample preparation

200 mg of the medicinal plant extract was dissolved in 2 ml of the methanol and then filtered through syringe filter (0.22µ). Finally prepared sample of each extract was loaded in GC-MS column.

#### 2.4.2 GC-MS analysis

GC MS analysis was carried out by splitless injection of 1µl of sample onto Shimadzu QP2010 GC-MS assembly was fitted with a column, coupled with mass detector. Following GS parameters were used during analysis of extract of medicinal plants. Column Oven Temperature was set at 100.0 °C, pressure was 175.1 kPa with total Flow of 16.3 ml/min, column flow was 1.21 ml/min, linear velocity was 28.9 cm/sec and purge flow was 3.0 ml/min. Mass detector was set with start time 6.00 min and end time 40.49min. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and WELLY library. The name, molecular weight and structure of the components of the test materials were ascertained.

### 2.5 Hydrogen peroxide scavenging assay

Antioxidative potential of ARE was determined by *in vitro* hydrogen peroxide scavenging assay. The ability of the ARE to scavenge hydrogen peroxide was assessed as per the method described by Ruch *et al.* [12]. The extent of H<sub>2</sub>O<sub>2</sub> scavenging by the plant extracts was calculated as per the following formula

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A<sub>0</sub> - Absorbance of control

A<sub>1</sub> - Absorbance in the presence of plant extract

## 3. Results

### 3.1 Percent Yield of ACE

5.23 gram of the hydromethanolic extract was recovered from 50 gram dry weight of leaves of *Acacia catechu*. Therefore the per cent yield was calculated to be 10.46 %.

### 3.2 Phytochemical analyses of ACE

As per the biochemical tests conducted, ACE shown presence of all the tested phytochemicals, viz. Resins, Saponin, Flavonoids, Alkaloids, Steroids, glycosides, Protein, Carbohydrate and Phenols (Table 1).

**Table 1:** Phytochemicals present in ACE

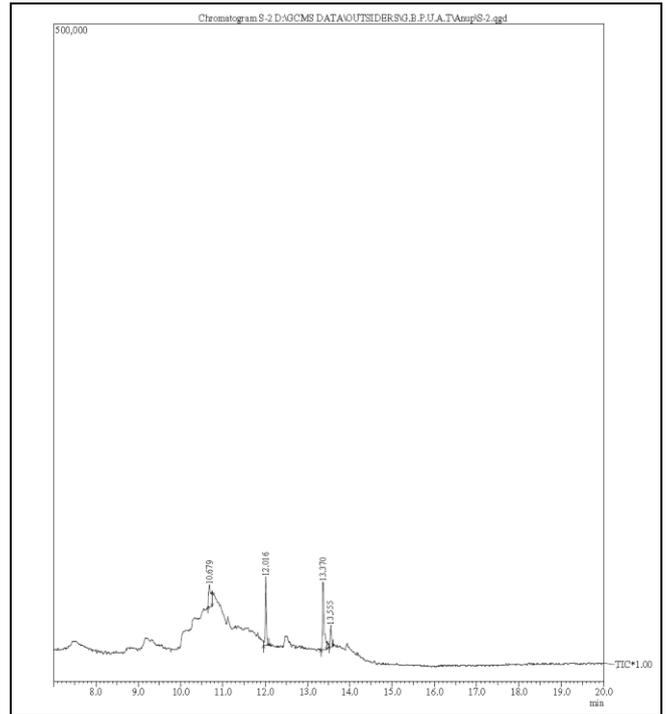
| S. No. | Phytoconstituents | ACE |
|--------|-------------------|-----|
| 1.     | Protein           | +   |
| 2.     | Carbohydrates     | +   |
| 3.     | Resins            | +   |
| 4.     | Tannins           | +   |
| 5.     | Saponins          | +   |
| 6.     | Flavonoids        | +   |
| 7.     | Alkaloids         | +   |
| 8.     | Steroids          | +   |
| 9.     | Phenols           | +   |
| 10.    | Glycosides        | +   |

### 3.3 GC-MS analysis of ACE

ACE was subjected to Gas chromatography and mass spectra analysis. GC-MS analysis of medicinal plant extract showed maximum hits with fatty acids. Phyto-constituents present in medicinal plant extracts were studied for their therapeutic properties.

ACE was subjected to GC-MS analysis with injection temperature of 270.00<sup>o</sup> C. the results are summarized in table 2 and chromatogram in figure 1. The composition of *Acacia catechu* showed presence of Caprylic acid Methyl Ester (19.77%), Lauric acid Methyl Ester (27.42%), 2-Ethyl-3-methyl-1-butene (42.09%) and Myristic acid Methyl Ester (10.72%); when chromatogram was compared with the TOX library. However, with NIST and WELLY library, the major constituent of *Acacia catechu* is predicted to be 6-

OCTADECENOIC ACID methyl ester (42.09%) (Fig. 1; Table 2 and 3).

**Fig 1:** Chromatogram showing peaks for phyto-constituents in ACE**Table 2:** Phyto-constituents present in ACE after determining the retention time peaks with TOX (PERFUMERY AND DRUG) Library.

| Peak | R.Time | Area   | Area% | Name                      | Formula  | CAS No.   | Mol.Wt. |
|------|--------|--------|-------|---------------------------|--|-----------|---------|
| 1    | 10.679 | 67867  | 19.77 | Caprylic acid ME          | C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>  | 111-11-5  | 158     |
| 2    | 12.016 | 94120  | 27.42 | Lauric acid ME            | C <sub>13</sub> H <sub>26</sub> O <sub>2</sub> | 111-82-0  | 214     |
| 3    | 13.37  | 144480 | 42.09 | 2-Ethyl-3-methyl-1-butene | C <sub>7</sub> H <sub>14</sub>                 | 7357-93-9 | 98      |
| 4    | 13.555 | 36803  | 10.72 | Myristic acid ME          | C <sub>15</sub> H <sub>30</sub> O <sub>2</sub> | 124-10-7  | 242     |
|      |        | 343270 | 100   |                           |  |           |         |

**Table 3:** Phytoconstituents present in ACE after determining the retention time peaks with NYST AND WELLEY Library

| Peak | R.Time | Area   | Area% | Name   | Formula  | CAS No.   | Mol.Wt. |
|------|--------|--------|-------|--|--|-----------|---------|
| 1    | 10.679 | 67867  | 19.77 | NONANOIC ACID, METHYL ESTER                  | C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> | 1731-84-6 | 172     |
| 2    | 12.016 | 94120  | 27.42 | HEXADECANOIC ACID, METHYL ESTER              | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> | 112-39-0  | 270     |
| 3    | 13.37  | 144480 | 42.09 | 6-OCTADECENOIC ACID, METHYL ESTER            | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> | 2777-58-4 | 296     |
| 4    | 13.555 | 36803  | 10.72 | TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> | 5129-66-8 | 256     |
|      |        | 343270 | 100   |  |  |           |         |

### 3.4 Hydrogen peroxide scavenging assay

The ability of ACE to scavenge hydrogen peroxide was assessed by calculating percent change in H<sub>2</sub>O<sub>2</sub> concentration

as displayed by absorbance in comparison to control. The percent H<sub>2</sub>O<sub>2</sub> scavenging activity of ACE was found to be 33.40% in comparison to control (Table 4).

**Table 4:** H<sub>2</sub>O<sub>2</sub> scavenging activity of ACE

| Treatments | Optical Density (O.D.) at 230 nm |       |       |       | Mean O.D.±S.E. | % H <sub>2</sub> O <sub>2</sub> scavenging activity |
|------------|----------------------------------|-------|-------|-------|----------------|---|
| Control    | 0.484                            | 0.489 | 0.52  | 0.46  | 0.488±0.0123   | —   |
| ACE        | 0.325                            | 0.32  | 0.356 | 0.299 | 0.325±0.0117   | 33.40   |

## 4. Discussion

*Acacia catechu* (Kath) has many nutritional properties and medicinal uses. The chief constituents of the heartwood and leaves of *Acacia catechu* are catechin and catechutannic acid [4]. The wood contains epicatechin, atzelchin, catechin tetramer, decatechin, gallochin, gossypectin, phlobatannin, kaempferol, quercitrin, quercitin and Taxifolin [13]. *Acacia catechu* contains catechuic acid, catechutannic acid (25%-33%), acacatechin (10%-12%), catechu red, quercetin, catechin (2%-12%), epicatechin, phlebotannin (25%-33%), gummy matter, quercitrin, quercitin and moisture. Quercitin is

a phenolic flavonoid and catechu of acacia is a pseudotannin. Catechu and epicatechin usually accompany other flavonoids [14]. Petroleum ether extract of *Acacia catechu* contain very high quantity of steroid and chloroform extract of *Acacia catechu* contain steroid, polyphenols and cumarin in average quantity. Ethyl acetate extract of *Acacia catechu* contain very high quantity of flavonoid and tannin as well as average quantity of quamarin and polyphenols [4]. Taxifolin has been reported to possess anti-fungal, antiviral, anti-inflammatory and anti-oxidative activity [16, 17]. Gulzar *et al* [15] did preliminary phytochemical and antimicrobial screening of

leaves extract of *Acacia catechu*. The extract of *Acacia Catechu* have been reported to have various pharmacological effects like immunomodulatory<sup>[18]</sup>, anti-pyretic, hypoglycaemic<sup>[19, 20]</sup>, anti-diarrhoeal<sup>[21]</sup>, antiulcerative<sup>[22]</sup> and hepatoprotective activity<sup>[21, 23]</sup>. The concentrated aqueous extract of *Acacia catechu* is an astringent, digestive, and is beneficial in cough and diarrhea<sup>[5]</sup>. Usually aqueous extract of bark of this plant is used in traditional herbal preparations.

According to one of the study conducted for the characterization of leaves extract of *A. catechu*, the purified form of extract was subjected to GC-MS analysis. The composition of *A. catechu* leaves extract had shown major components as terpene *i.e.* camphor (76.40%) and phytol (27.56%) along with other terpenes in minor amounts which are related with their high antibacterial and antifungal properties<sup>[24]</sup>. Saha *et al.*<sup>[25]</sup> reported that leaf extract had significant antioxidant activity against various free radicals. They further suggested that the pronounced antioxidant activity of the extract was due to its phenolic and flavonoid content. The chemical characterization of the extract using NMR and GC-MS revealed presence of several antioxidative compounds.

In another study, *Acacia catechu* showed very high antioxidant activity, probably due to the high polyphenol content. Rat liver post mitochondrial supernatant (PMS) in Tris HCl buffer, pH 7.4 was incubated for 0 and 1 hr, with various test extracts in three different oxidant systems. The results showed that the addition of test samples to FeCl<sub>3</sub> medium at 0 hr significantly stopped the initiation of the LPO. However, the propagation phase of LPO was inhibited by *Acacia spp.* The test samples also showed strong reducing power and superoxide radical scavenging activity of *Acacia catechu*<sup>[26]</sup>. Hédi, *et al.*,<sup>[27]</sup> indicated that extracts of *Acacia salicina* leaves are a significant source of compounds with anti-genotoxic and antioxidative activity (most likely phenolic compounds and sterols) and thus may be useful for chemoprevention. They reported that crude extracts of leaf possess least reducing ability as compared to bark, and heartwood of *A. catechu*. Stohs and Bagchi<sup>[28]</sup> reported that *A. catechu* heartwood extracts possess antioxidant, anti-inflammatory and chemo-protective properties. Thus it could be inferred from the present study that the presence of various phytochemicals as revealed through biochemical and GC-MS analyses may be responsible for antioxidative potential of *A. catechu* leaf extract. However, advance analysis is required to further harness the medicinal potential of *A. catechu*.

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