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Analysis of Fatty Acids in Acacia Catechu Seed Extract by Gas Chromatography Mass Spectrometry (Gcms)

Running title: Analysis of fatty acids in acacia catechu seed extract by gas chromatography mass spectrometry (GCMS)

Sindhu priya Kuppusamy
Undergraduate
department of pharmacology
Saveetha dental college
Saveetha university
chennai, 600077, Tamil Nadu, India.

Lakshmi Thangavelu
Assistant Professor of department of pharmacology
Saveetha dental college
Saveetha university
Chennai 600077 , Tamil Nadu, India.

Corresponding author
Lakshmi Thangavelu
Assistant Professor of department of pharmacology
Saveetha dental college
Saveetha university
chennai, 600077, Tamil Nadu, India.
Emails I'd: Lakshmi085@gmail.com

ABSTRACT

INTRODUCTION: Acacia seeds belongs to the family Leguminosae. Whereas, ACACIA catechu belongs to Fabaceae family. They posses various properties, such as; antioxidant, anti-cancer, anti-haemolytic, anti-inflammatory, anti-pyretic, analgesics and anti-depressant. It is used to treat, against, fever, malaria, cholera, dysentery, and even oral thrush. The many

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functions are due to the presence of flavonoids, called catechin. And polyphonic compounds such as; Rutin, caffeine acid, etc.

MATERIALS AND METHOD:

3 standard preparations were made with palmitic, linolenic and stearic acid, to calculate the fatty acid levels in acacia seeds using GCMS analysis.

RESULTS: The amount of fatty acids formed were substantial and showed that we can proceed extracting from Acacia seeds.

CONCLUSION: Fatty acids extracted from Acacia seeds can be used for various properties, such as; anti-inflammatory, malaria, fever, oral thrush, etc.

KEYWORDS: Acacia catechu, medicinal plant, fatty acids, polyphenolic compounds, linolenic acid, stearin acid, Palmitic acid.

INTRODUCTION

Acacia seeds belongs to family Leguminosae[1] possesses antioxidant, anticancer, anti-haemolytic, anti-inflammatory, antipyretic, analgesic and antidepressant potentials. *Acacia catechu* Willd (*Fabaceae*), commonly known as catechu, cachou, and black cutch, is a moderate size deciduous, thorny tree widely distributed in India.

The name of the plant has recently been changed to *Vachellia karroo*[2]. The gum produced by *A. karroo* is used against oral thrush and can also be harvested for food during hard times. *Acacia* is also effective against fever, malaria, cholera, diarrhoea, dysentery and high blood pressure. *Acacia s*pecies are rich sources of polyphonic compounds, known to have strong antioxidant properties that help in the prevention of various oxidative stress. These activities might attribute to the presence of various active secondary metabolites i.e. gallic acid, catechin, rutin, caffeic acid, 7-*O*-galloyl catechin, +catechin and methyl gallate. Flavonoids, a type of water-soluble plant pigments, are the major class of compounds isolated from *Acacia* plants. Catechin is a major flavan in *Acacia* bark and heartwood, found primarily in green tea.

Various parts of this plant have been used since ancient times in Ayurvedic medicine.[3]Numerous natural bioactive compounds for instance 4-hydroxybenzoic acid, kaempferol, quercetin, 3,4,7-trihydroxyl-3,5-dimethoxyflavone, catechin, rutin, isorhamnetin,

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epicatechin, afzelechin, epiafzelechin, mesquitol, ophioglonin, aromadendrin, and phenol have been isolated from heartwood, bark, roots, leaves and stem of *A. catechu* and presence of the above active compounds have been implicated for its myriad biological effects. The phytochemical isolated from this plant have been widely studied for their cytotoxic potentials against variety of cancer cell lines and came out with good results[4,5,6].*A. catechu* has been studied for its hepatoprotective, antipyretic, antidiarrheal, hypoglycaemic, anti-inflammatory, immunomodulatory, antinociceptive, antimicrobial, free radical scavenging, and antioxidant activities.

Extensive animal in vivo studies and human clinical trials compositions containing *Acacia* extract indicate that *Acacia* has great potential as a therapeutic agent for inflammatory diseases such as arthritis, irritable bowel syndrome, and inflammatory bowel syndrome[7].Catechu black extract has been approved by the US FDA for food use as a natural flavouring substance and/or natural substance used in conjunction with flavour.

Fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28.Fatty acids are usually derived from triglyceridesor phospholipids. Two essential fatty acids are linoleic acid(LA) and alpha-linolenic acid (ALA)[8].These fatty acids are widely distributed in plant oils. The human body has a limited ability to convert ALA into the longer-chain omega-3 fatty acids—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can also be obtained from fish. Omega-3 and omega-6fatty acids are biosyntheticprecursors to endocannabinoidswith antinociceptive, anxiolytic, and neurogenic properties.

Medicinal plants are currently of considerable importance because of their fatty acids which has potential therapeutic value that lead them to the path of development of novel drugs. Presence of beneficial fatty acids and the shift towards natural products in pharmaceutical and cosmeceutical industry made medicinal plant research equally important to conventional drug. methods like conventional solvent extraction, steam distillation, and sublimation, etc., are developed for extraction fatty acids.[9]However, these methods are based on sequential extraction, including one or more organic solvents. Such phytochemical extracts need to be processed for the removal of traces of the organic solvents.

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Furthermore, the mixture has to be purified for individuality. While such methods are useful for extraction and purification of small quantities of fatty acids for research purposes, completely removing the organic solvents from the extracts is a problematic issue. Furthermore, the types and concentrations of organic solvents must be carefully selected to avoid structural changes to the target phytochemical during extraction. Such changes adversely affect one or more of their desirable physical, chemical, and biological properties. Water, is an inexpensive, environment- friendly and an ideal solvent for the industrial extraction of medicinal plants, but its use is limited due to poor extraction efficiency for most organic compounds. The aim of this study is to assess and determine the amount of fatty acids in acacia seed extract.

MATERIALS AND METHOD:

PLANT MATERIAL COLLECTION AND EXTRACTION

Acacia catechu seeds were obtained from Hosur in Tamil Nadu and were processed by Green Chem lab in Bangalore.

PREPARATION OF ACACIA EXTRACT

Seeds were shade dried for a week. Dried seeds were milled to one powder. Powder was passed through 100 mesh sieve and stored in a sealed poly- thene bag 2.5 kg of powdered Acacia catechu seeds were extracted with 10 liters of Ethanol, at 65°C temperature, for 1 hour, in a 20 liter round bottom flask with Graham condenser attached. Condenser was cooled circulating with chilled water. After 1 hour of extraction, round bottom flask was cooled to room temp and the extract were altered and collected. e Marc was extracted repeatedly with 10 liters of Ethanol, twice. The extracts were altered and collected. The combined extracts was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C, to obtain 150 g of powder extract. The w/w yield of the prepared extract was 6%. The extract were stored at 4°C until used.

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CHEMICALS AND REAGENTS

DPPH, BHA, Alpha glucosidase, Folin-Ciocalteu, Alpha amylase, Tris-Hcl bu er, P-nitro phenyl- glucopyranoside, Tryp- sin, starch,dinitrosalicylic acid, were procured from Sigma Chemicals , USA. Other re- agents like sodium carbonate perchloric acid,dimethyl sulfoxide (DMSO), sulphuric acid, ammonium molybdate and sodium phosphate were purchased from Merck, India .

Methodology:

Column Name: Elite-WAX; size: 30m (a film thickness of 1µm df),Inner diameter =0.32 mmID
Carrier gas : Helium (He)
Flow rate : 1.0 ml/min
Split ratio : 1:10
Injection Port : 250°C
Detection : EI- Mass (Temperature: Ion source-230°C; Interface- 250°C)
Column Oven : 80°C

Table 1: Column Temperature Program:

Rate (C/min)	Temperature	Hold Time (min)
------	80.0	1
10.0	220.0	20

Standard Preparation 1: Weigh accurately about 50mg of Stearic acid (Stearic acid/Ref/01) in a clean Round bottom flask fitted with reflux condenser. Add 5ml of Boron triflouride solution and reflux for 15 minutes until the solid is dissolved. Cool, transfer the reaction mixture with the aid of 10ml of Hexane in a separator and add 10ml of water and 10ml of saturated Sodium chloride solution. Shake well and allow the layers to separate then drain and discard the lower aqueous layer. Pass the hexane layer through anhydrous Sodium sulphate into a suitable flask.

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Standard Preparation 2: Weigh accurately about 50mg of Palmitic acid (Palmitic acid/Ref/01) in a clean Round bottom flask fitted with reflux condenser. Add 5ml of Boron triflouride solution and reflux for 15 minutes until the solid is dissolved. Cool, transfer the reaction mixture with the aid of 10ml of Hexane in a separator and add 10ml of water and 10ml of saturated Sodium chloride solution. Shake well and allow the layers to separate then drain and discard the lower aqueous layer. Pass the hexane layer through anhydrous Sodium sulphate into a suitable flask.

Standard Preparation 3: Weigh accurately about 50mg of Linoleic acid (Linoleic acid /Ref/01) in a clean Round bottom flask fitted with reflux condenser. Add 5ml of Boron triflouride solution and reflux for 15 minutes until the solid is dissolved. Cool, transfer the reaction mixture with the aid of 10ml of Hexane in a separator and add 10ml of water and 10ml of saturated Sodium chloride solution. Shake well and allow the layers to separate then drain and discard the lower aqueous layer. Pass the hexane layer through anhydrous Sodium sulphate into a suitable flask.

Sample Preparation: Weigh accurately about 200mg of Acacia catechu seed extract in a clean Round bottom flask fitted with reflux condenser. Add 5ml of Boron triflouride solution and reflux for 15 minutes until the solid is dissolved. Cool, transfer the reaction mixture with the aid of 10ml of Hexane in a separator and add 10ml of water and 10ml of saturated Sodium chloride solution. Shake well and allow the layers to separate then drain and discard the lower aqueous layer. Pass the hexane layer through anhydrous Sodium sulphate into a suitable flask.

Boron triflouride preparation: weigh accurately 14gm of Boron triflouride in a clean conical flask. Add 50ml of Methanol slowly. Mix well and make upto 100ml with Methanol.

Inject 1.0ul of Standard and Sample separately. Calculate the area of the Standard as compared to sample and calculate the assay.

Calculations:

1. **Calculation of Stearic acid in *Acaica catechu* seed extract**

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Standard batch No:Stearic Acid/Std/Ref 01/97.6%

Weight of the Std:50.4mg

Standard Area:17422832

Sample Batch No:Acacia catechu seed extract

Weight of the sample:200.5mg

Sample Area:249495

Extraction solvent:Boron triflouride solution

Std Assay:97.6%

Calculation:

(50.4 / 200.5) X (249495/ 17422832) X 97.6% = 0.35%

2. **Calculation of palmitic acid in *Acaica catechu* seed extract**

Standard batch No:PalmiticAcid/Std/Ref 01/98.8%

Weight of the Std:57.6mg

Standard Area:98551566

Sample Batch No:Acacia catechu seed extract

Weight of the sample:200.5mg

Sample Area:1280956

Extraction solvent:Boron triflouride solution

Std Assay:98.8%

Calculation:

(57.6 / 200.5) X (1280956/ 98551566) X 98.8%= 0.36%

3. **Calculation of Linoleic acid *Acaica catechu* seed extract**

Standard batch No:Linoleic Acid/Std/Ref 01/75%

Weight of the Std:50.8mg

Standard Area:26727141

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Sample Batch No:Acacia catechu seed extract

Weight of the sample:200.5mg

Sample Area:990825

Extraction solvent:Boron triflouride solution

Std Assay:97.6%

Calculation:

(50.8 / 200.5) X (990825/ 26727141) X 75% = 0.70%

RESULTS:

Sample Information

Analyzed by	: Admin
Analyzed	: 12/9/2014 12:34:35 PM
Sample Name	: Acacia catechu seed extract
Sample ID	: Acacia catechu seed extract
Vial #	: 2
Injection Volume	: 1.00
$EndIf$Data File	: D:\GCMS DATA\Data\FT_29102014\Fatty Acids 17.qgd
Method File	: D:\GCMS DATA\Method\Fatty Acids scan.qgm
Report File	: D:\GCMS DATA\Report\Default.qgr
Tuning File	: D:\GCMS DATA\Tune\Fatty acids tune CID gas off.qgt

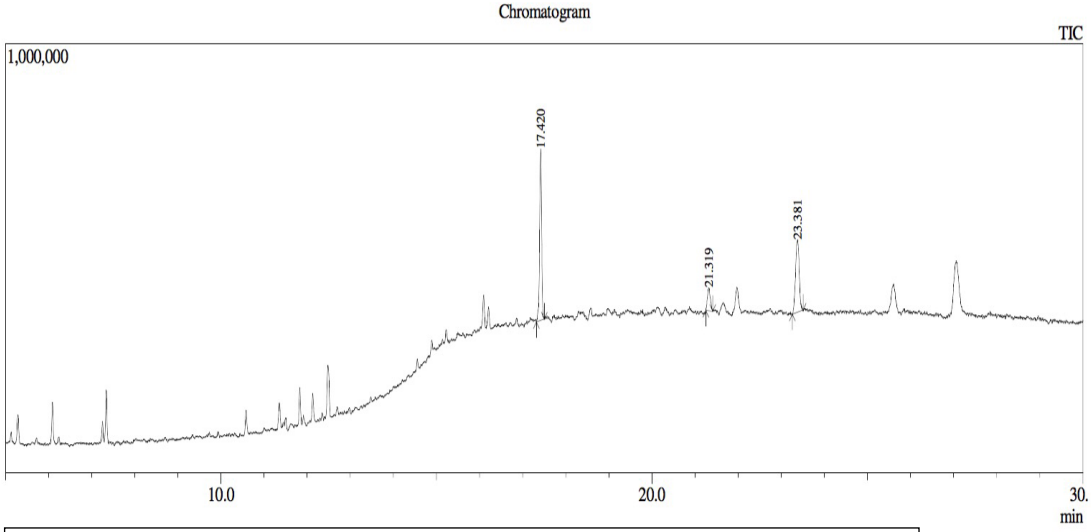

Table 2: peaks showing fatty acid level in *Acacia seeds,* using GCMS

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	Name
1	17.420	17.320	17.505	1280956	50.81	394908	64.20	Methyl palmitate
2	21.319	21.250	21.415	249495	9.90	52896	8.60	Methyl stearate
3	23.381	23.260	23.510	990825	39.30	167304	27.20	Methyl linoleate
				2521276	100.00	615108	100.00	

Figure 1 : Results by GCMS

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DISCUSSION

Antioxidant molecules are gaining lot of interest in this respect. Antioxidants are also attracting the attention of the scientific community because of their potential to be used as a dug and supplement to drug in case of many degenerative diseases to minimise the complications. Synthetic antioxidant like butylatedhydroxy anisole (BHA) or butylatedhydroxy toluene (BHT) or propyl gal-late (PG) are supplemented to reduce the oxidative damage but due to side effect these are not well accepted antioxidant.[10] It is generally assumed that frequent consumption of plant-derived phytochemical from vegetables, fruit, tea and herbs may contribute to shift of balance towards an adequate antioxidant status[11]. The extraction of these bioactive phytochemical uses organic solvents but the organic solvents lead to the issue of safety and acceptability by human body.

A.catechu has a high therapeutic value as it helps to relieve the symptoms of diseases like lymphatic and venous insufficiency, hemorrhagic diseases, and hypertension. A.catechu was found to induce apoptosis in LNCaP cells (Prostate cells) at low concentration and in HepG2 cells (human hepatoma) at very high concentration by acting as pro-oxidant instead of an antioxidant which resulted in programmed cell death. It inhibited the tumour cell growth by blocking cells amelioration through G0-G1 transition which is medicated by down regulation of cyclin D1 and E. A.catechu also found to exhibit excellent antibacterial property against *E. coli* strain (incorporated with envA1 allele) by inhibiting the action of enzyme type II topoisomerase as well as its selective promotion of *E. coli* topoisomerase IV-dependent DNA cleavage which is very essential for the survival of the cell.

Further, *A. catechu* is proven for its cytotoxic potentials against various cell lines.[12]These findings suggest the fact that active phytochemical components present in *A. catechu* are responsible for the mechanism by which they induce cytotoxicity and our current results are in agreement with these reports. Staining of apoptotic cells with fluorescent dyes such as AO/EB is considered one of the methods for evaluating the nuclear morphology changes.[13,14]Previous studies have performed AO/EB staining and reported that early apoptotic cells had fragmented DNA which exhibited intense green coloured nuclei. Late apoptotic and necrotic cells DNA were fragmented and stained orange and red.[15] In light of

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the above reports, the presence of high orange stain intensity in cells treated with high concentration of ACB extract further confirm the DNA fragmentation and apoptosis.

A.catechu has been previously reported for its anti-HIV activity.[16] The present authors have previously demonstrated that *A. mellifera*ethyl acetate, n-butanol and aqueous extracts also have hepatoprotective and anti-HBV effects. [17] In the present study, anti-HBV evaluation of *A. oerfota* extracts at a non-cytotoxic dose showed its association with the methanol and aqueous extracts. Furthermore, the highest anti-HBV activity of *C. grandis* and *C. epigeus*was associated with the crude ethanolic-extract, indicating the possibility of synergy among the antiviral phytochemical constituents of the extract. Synergistic activity of antiviral components of plant extracts that act by different mechanisms has been reported previously.[18]

The antimalarial activity of *A. Catechu* extracts (leaves, pods and bark) were determined by the schizont maturation inhibition assay. Ethanol extract S of leaves, pods and bark were screened for antimalarial activity against an artesunate sensitive strain of *P. falciparum*(3D7). The schizonticide activities were expressed in terms of IC_{50} values, defined as minimum concentration of plant extracts required to inhibit 50% of schizont maturation. The schizont maturation inhibition results at 48 h and 96 h by artesunate, leaves, pods and bark extracts. After 48 h of incubation, leaves were found more potent than pods and bark against the plasmodium parasite with an IC_{50} of 1.29 (1.08–1.49) μg/ml.[20]

CONCLUSION:

Fatty acids extracted from Acacia seeds can be used for various properties, such as; anti-inflammatory, malaria, fever, oral thrush, etc.

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Table legends:

Table 1 –column temperature program

Table 2 - peaks showing the fatty acid level in *Acacia seeds*, using GCMS analysis

Figure 1 - peaks showing the fatty acid level in *Acacia seeds*, using GCMS analysis