Flavanoids profile of different Acacia catechuWildFractions by HPTLC Densitometric Method of Analysis

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Abstract:

Since ancient times, acacia catechu has been used in the traditional "Ayurvedic" medicine of India. But no attempt has been made to standardise.In terms of active ingredient or marker, the herbal extract serves as the major component. A chromatographic fingerprint is used to depict the therapeutically desirable chemical components of herbal medicines. This study suggests an exact HPTLC approach for the quantitative assessment of flavonoids like catechin, kaempferol, quercetin, and taxifolin from water, ethyl acetate, and butanol fractions of Acacia catechu extract. According to the research, catechin and taxifolin, two flavanoids, are present in the water fraction of the extract in amounts of 17.80% and 3.48%, respectively. Catechin, kaempferol, and quercetin have respective concentrations in the butanol fraction of the Acacia catechu extract of 15.48%, 3.04%, and 11.79%. Catechin, taxifolin, kaempferol, and quercetin were all present in the ethyl acetate fraction of the Acacia catechu extract at amounts that were, respectively, 28.61%, 1.51%, 2.62%, and 12.32%. The ethyl acetate fraction of Acacia catechu is plentiful in

flavanoids since it had a higher total flavonoid content (41.66 mg QE/gm) than the water (11.66 mg QE/gm) and butanol fractions (33.33 mg QE/gm) of the extract.

Key words: Acacia catechu, catechin, kaempferol, quercetin, taxifolin, protocatechuic acid, gallic acid, HPTLC.

Introduction

Although India has been using Ayurveda as a system of healthcare for more than 6000 years, the industry's expansion just started in recent years. About 10% of medicines are exported globally from India, which is a small percentage. As a result, it is necessary to make Ayurveda into a vibrant sector that is grounded in science and draws on the extensive body of knowledge of ancient tradition. [1, 2]Medium-sized deciduous Acacia catechu Wild, often referred to as Khair, with a forked and crooked trunk. In most regions of the nation, it is observed to be growing in both natural and planted forms [3]. The greater variety of therapeutic characteristics of Acacia catechu are used in traditional medicine. The tree's heartwood, bark, and leaves are only a few of the parts of medicinal value. Medium-sized deciduous Acacia catechu Wild, often referred to as Khair, with a forked and crooked trunk. In most regions of the nation, it is observed to be growing in both natural and planted forms [3]. Because of its extensive array of therapeutic characteristics, acacia catechu is utilised in traditional medicine. The tree's heartwood, bark, and leaves are only a few of the parts of therapeutic potential.[4] Because of its potent astringent and antioxidant properties, acacia catechu is very valued. It helps with dental, oral, and throat infections as well as acts as an astringent to lessen seeping from open wounds and chronic ulcers. A variety of pharmacological properties, including antimicrobial, hepatoprotective, anti-inflammatory, anti-diarrheal, anti-pyretic, and anti-inflammatory properties, are displayed by acacia catechu preparations. [5-9] Catechin, (-) Epicatechin, Epigallocatechin, Epicatechingallate, Protocatechuic acid, Phloroglucin, Poriferasterolglucosides, Poriferasterolacyglucosides, Lupenone, Kaempferol, Taxifolin, (+)-Afzelchin, and minerals are the main chemical components of *Acacia catechu Wild.*[10-15] Acacia is one of the plants that is particularly high in flavonoids and contains a variety of flavonoids that are crucial for many biological processes. Despite this estimation, the phenolics, tannins, and flavonoids in the Acacia catechu hydroalcoholic, ethyl acetate, and butanol Fractions of the plant have not been explored.

As a result, this study was designed to qualitatively as well quantitatively analyze the flavonoids, and phenols in thesefractions. Different mobile phases are used for flavonoids, phenols,

Material and Method:

Plant Materials:

The plant's heartwood was harvested in February 2022 from the Akluj district of Solapur, Maharashtra, India. The Department of Botany at DBF Dayanand College of Arts and Science in Solapur then verified its authenticity.

Preparation of plant extract:

The powdered Katha that was extracted using a soxhlet method with 10% Hydro-alcoholic solution. Hydro-alcoholic extract dried (yield 27%) and dissolved in water and water is evaporated with rotary evaporator to obtain dry water Fraction (Yield 17%)For ethyl acetate fraction hydroalcholic extract dissolve in ethyl acetate and then evaporate to dry with rotary evaporator to obtain dry ethyl acetate fraction (11.5%), For Butanol fraction hydroalcholic extract dissolve in butanol and then evaporate to dry with rotary evaporator to obtain dry Butanol fraction (8%)

Preliminary phytochemical screening of extract:

Acacia catechu extract was subjected to preliminary phytochemical screening, which revealed the presence of tannins, flavonoids, saponins, and triterpenes [17], as indicated in Table 1.

Quantitative phytochemical screening

Estimation of total flavonoid content:

Using a colorimetric aluminium chloride assay, the total flavonoid concentration was determined. The reaction mixture is composed of 1 ml of water , ethyl acetate and butanol portion of Acacia catechu extract and 4 ml of distilled wate in a 10 ml volumetric flask, add 0.30 ml of 5% sodium nitrite. After five minutes add 0.3 ml of 10% aluminium chloride. After waiting for five minutes,add 2 ml of 1M sodium hydroxide was treated and diluted with distilled water to make 10 ml. Similar reference standard quercetin solutions (200, 400, 600, 800, and 1000 g/ml) were made. An UV-Visible spectrophotometer was used to measure the absorbance

of the test and standard solutions at 510 nm in comparison to the reagent blank. In mg of quercetin equivalents, the total flavonoid content was calculated[18].

HPTLC analysis of fractions of *Acacia catechu wild*:

Method, Chemicals and Reagents

Markers like Catechin, Taxifolin, Kaempferol, Quercetin, procured from YUCCA Enterprises, Mumbai.Ethyl acetate, n-Butanol, and Ethanol [all Reagents of analytical grade, E-Merck]. Apparatus Linomat IV automatic sample spotter with a CAMAG (Muttenz, Swizerland) TLC chamber: Glass twin trough chamber (20× 10× 4).Syringe: 3µL sample & 5µL Standard Hamilton syringe (Bonadug, Swizerland) Plate: 10×10cm, 0.2mm precoated with silica gel 60F254, Densitometer: TLC scanner 3 with SPI software 1.21 version; CAMAG HPTLC;

Preparation of standard Solution

1. Catechin :Weighed 5.469 mg of catechinand add methanol. Sonicate and dilute to 5 ml with methanol
   Mobile Phase :Toluene :Ethyl acetate:Formic Acid (30:30:6) [20]
2. Taxifolin : Weighed 0.349 mg of taxifolin and add methanol. Sonicate and dilute to 5 ml with methanol
   Mobile Phase: Mobile Phase: Chloroform: Ethyl acetate: GAA (4:4:2)
3. Kaempferol : Weighed 2.118 mg of kaempferol and add methanol. Sonicate and dilute to 10 ml with methanol.
   Mobile Phase: Chloroform: Ethyl acetate: GAA (4:4:2)
4. Quercetin :Weighed 5.070mg of quercetin add little quatity of methanol and dilute to 10 ml with methanol.
   Mobile Phase: Chloroform:Ethylacetate:Glacial acetic acid (4:4:2) [19]

Preparation of sample Solution:

1. BE: Weighed 5.469 mg of BE sample and add methanol to dissolve the sample. Sonicate and dilute to 1ml with methanol

2. EAE: Weighed 5.004 mg of EAE sample addadd methanol to dissolve the sample. Sonicate and dilute to 1ml with methanol
3. WE:Weighed 5.008 mg of EAE sample addadd methanol to dissolve the sample. Sonicate and dilute to 1ml with methanol [19]

Mobile phase: For catechin:Toluene :Ethyl acetate:Formic Acid (30:30:6), for Taxifolin: Chloroform: Ethyl acetate: GAA (4:4:2), Kaempferol: Chloroform: Ethyl acetate: GAA (4:4:2), Quercetin: Chloroform:Ethylacetate:Glacial acetic acid (4:4:2)

Spot development:

After being saturated with solvent vapour for 20 minutes, the sample-loaded plate was placed in a TLC twin trough developing chamber along with the appropriate mobile phase, and the plate was developed up to 90 millimetres.**Photo-documentation:** The developed plate was dried using hot air to evaporate the solvents from the plate. The plate was kept in a Photo-documentation chamber (CAMAG REPROSTAR 3) and the images captured at 288nm, 210nm, &277nm

Scanning: The plate was positioned in the scanner stage (CAMAG TLC SCANNER 3 "Scanner3 170308" S/N 170308(1.14.30)) and scanned at wavelengths of 288nm, 210nm, and 277nm. Peak densitogram, Peak table, and Peak display were noticed.

Result and Discussion:

Preliminary phytochemical screening:

The presence of tannins, flavonoids, triterpenes, glycosides, and steroids was shown by a preliminary phytochemical study of the water, ethyl acetate, and butanol fractions of Acacia catechu extract. As indicated in Table 1, the ethyl acetate fraction of Acacia catechu contains more flavonoids and triterpenes than the water and butanol fraction.

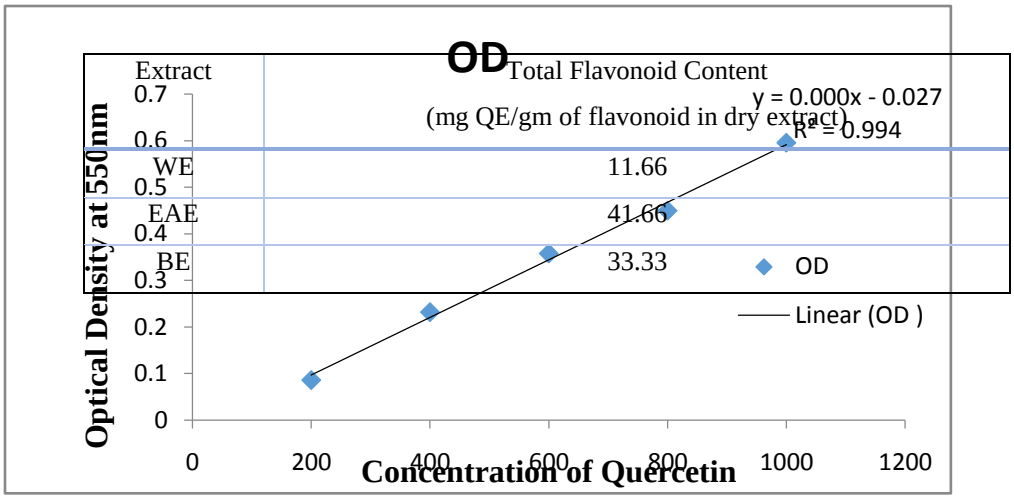
Table 1: Preliminary phytochemical screening of water, ethyl acetate and Butanol fractions of Acacia catechu Extract

Sr.no.	Metabolite	Test	Hydroalcoholic Extract	Ethyl acetate Extract	Butanol Extract
1.	Tannins	Gelatin	++	++	++
2.	Flavonoids	Lead acetate, Alkaline reagent	+	+++	++
3.	Triterpenes	Liebermann burchard	++	+++	+
4.	Glycosides	Modified borntagers	+	++	+
5.	Saponins	Froth formation	-	-	-
6.	Steroids	Salkowski	+	+	+
7.	Alkaloids	Mayers and wagner reagent	-	-	-

Total Flavonoid Content

The most prevalent and widely present class of plant phenolic chemicals, flavonoids are distinguished by a benzo-pyrone structure. They are present in almost every fruit and vegetable. By reacting with sodium nitrite, the total flavonoid concentration of the sample fractions can be ascertained. With a maximum wavelength of 510 nm, spectrophotometry can be used to track the formation of a coloured flavonoid-aluminum complex using aluminium chloride in an alkaline environment. With a coefficient of determination (R2) value of 0.9949, a linear calibration curve for quercetin, used as a standard, was obtained, as shown in Figure: 2. The Table:2 lists the total flavonoids in the water fraction, ethyl acetate fraction, and butanol fractions. More flavonoids (41.66 mg QE/gm) are present in the ethyl acetate Fraction of Acacia catechu than in the water (11.66 mg QE/gm) or butanol (33.33 mg QE/gm) fractions.

Table 2: Total Flavonoid Content of WE, EAE,BE of Acacia Catechu



Graph 1: Total Flavonoid Content of WE, EAE, BE of Acacia Catechu

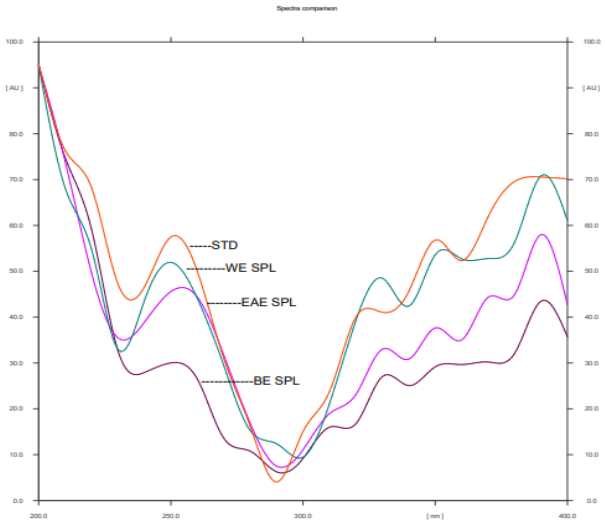
HPTLC profile of Acacia catechu fractions

Acacia catechu has a number of flavonoid phytoconstituents, four of which were quantified using the dentiometric method of HPTLC. The flavonoids were scanned at wavelengths of 288 nm, 210 nm, and 277 nm for the water fraction, ethyl acetate fraction, and butanol fraction. As markers, pure catchin, quercetin, taxifolin, and kaempferol were utilised. Studying the concentrations of catechin, quercetin, Taxifolin, and Kaempferol in the water fraction, ethyl acetate fraction, and butanol fraction of Acacia catechu Extract revealed that catechin sis present in Water fraction (17.80%) , ethyl acetate fraction (28.61%) and butanol fraction (15.18%) as shown in Graph 2 to graph7. The Taxifolin is present in water fraction (3.48%) and ethyl acetate fraction (1.51%) only and is absent in butanol fraction.as shown in Graph 8 to graph 13. The kaempferol is absent in water fraction and present in ethyl acetate fraction (2.62%) and butanol fraction (3.04%) as shown in graph14 to graph 19 . The Quercetin is abent in water fraction and is present in ethyl acetate fraction (12.32%) and butanol fraction (11.73%) as shown in graph 20 to graph 25. catechin was present in the water fraction at a rate of 17.80% with an Rf value of 0.55 and Taxifolin was present at a rate of 3.48% with an Rf value of 0.68, but quercetin and Kaempferol were not present as shown in graph .The butanol fraction scanned at 288 nm has a catechin content of 15.18% with an Rf value of 0.58, kaempferol content of 3.04% with an Rf value of 0.84, quercetin content of 11.79% with an Rf value of 0.83, and taxifolin is not present. Catechin is present in the ethyl acetate fraction scanned at 288 nm with an Rf value of 0.55, while taxifolin is

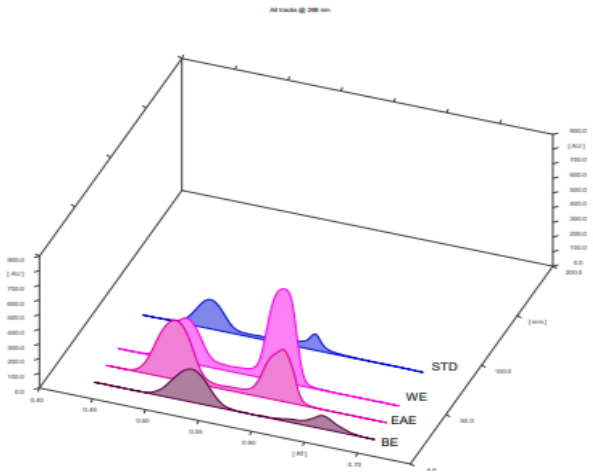
present with an Rf value of 1.51%. containing quercetin 12.32% with an Rf value of 0.83 and kaempferol 2.62% with an Rf value of 0.83, which was noted in table no 3. Fig. No. had the relevant HPTLC chromatogram.

Table3: Quantitative Estimation of Marker in Water fraction, Ethyl Acetate fraction & Butanol fraction

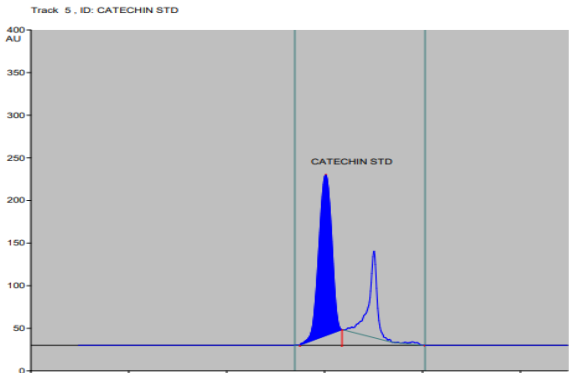
Sr. No	**Name of Marker**	**Concentration**		
		WE	**EAE**	**BE**
1.	Catechin	17.80%	28.61 %	15.18%
2.	Taxifolin	3.48%	1.51%	-
3.	Kaempferol	-	2.62%	3.04%
4.	Quercetin	-	12.32%	11.79%



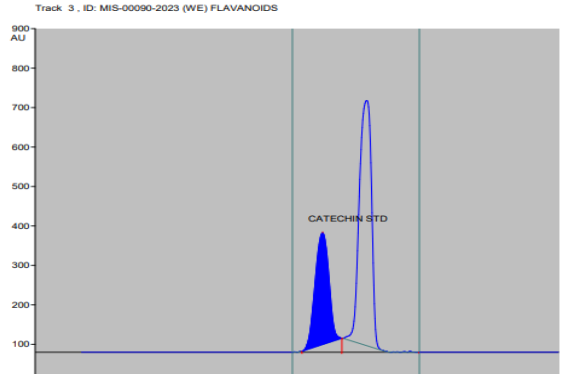
Graph 2: Spectra of Catechin showing concentration inWE, EAE, BE



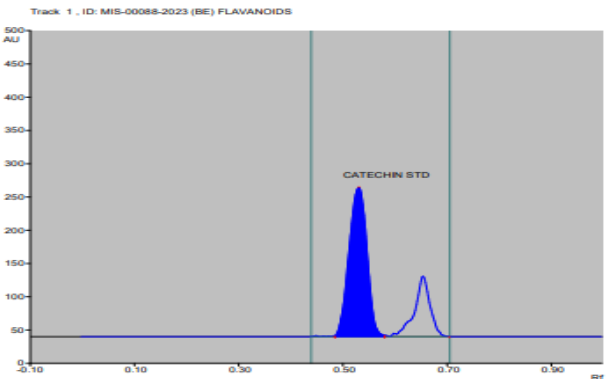
Graph 3: Dentiogram showing concentration of catechin inWE, EAE, BE



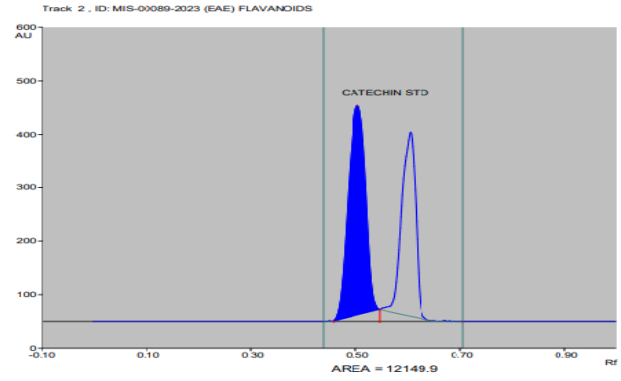
Graph 4: Standard Catechin



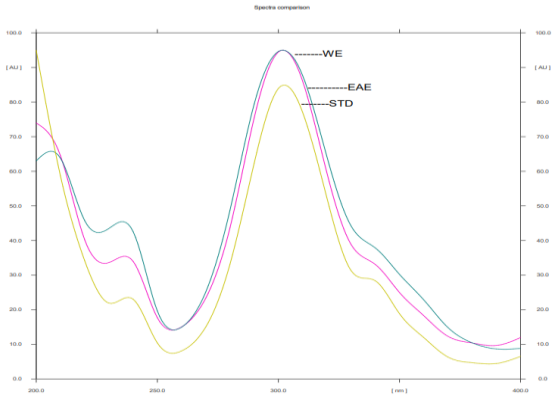
1. **Graph 5: Catechin in Water Extract (WE)**



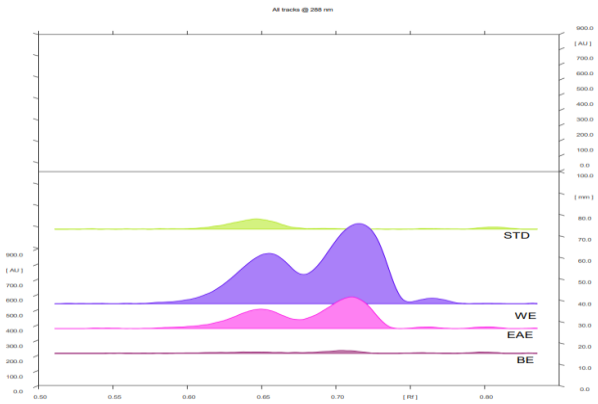
Graph 6: Catechin in Butanol Extract (BE)



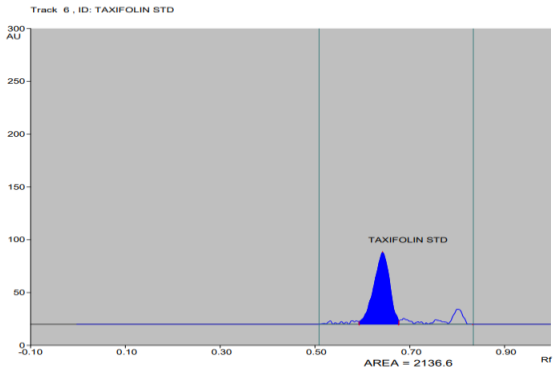
Graph 7: Catechin in Ethyl acetate extract Extract (EAE)



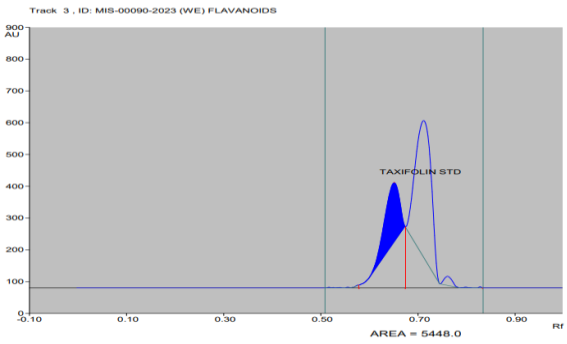
Graph 8: Spectra of Taxifolin showing concentration in WE, EAE, BE



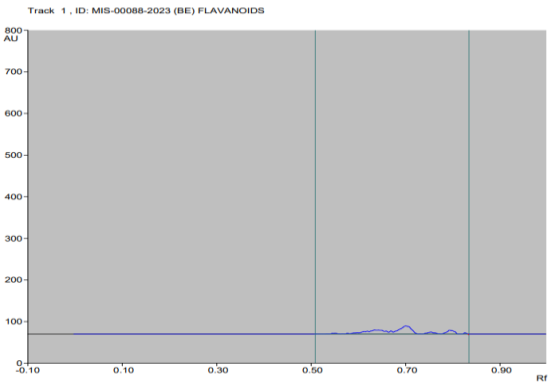
Graph 9: 3D View of Taxifolin showing concentration in WE, EAE, BE



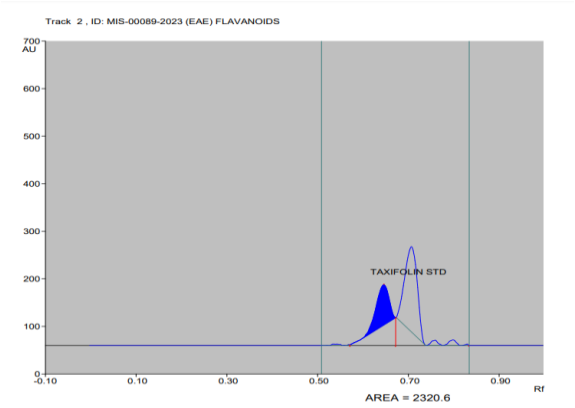
Graph 10: Standard Taxifolin



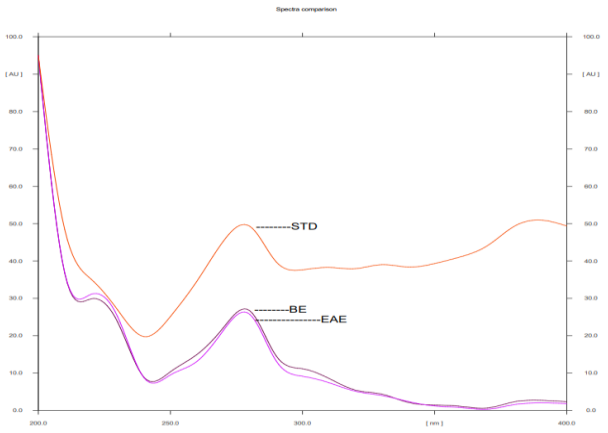
Graph 11: Taxifolin in Water Extract (WE)



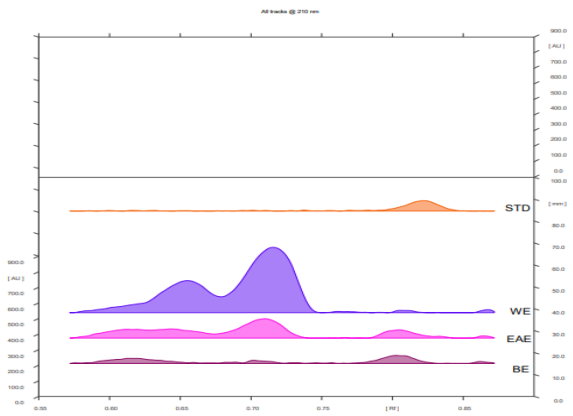
Graph 12: Taxifolin in Butanol Extract (BE)



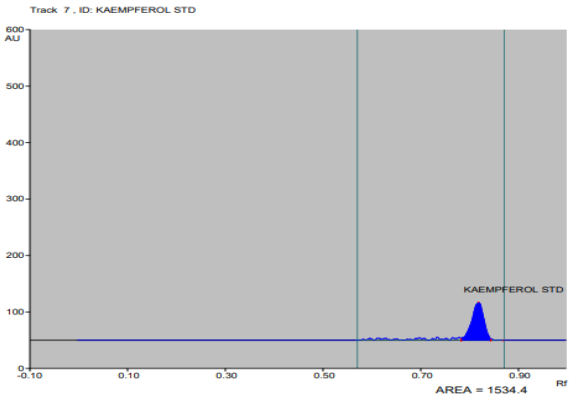
Graph 13: Taxifolin in Ethyl acetate Extract (EAE)



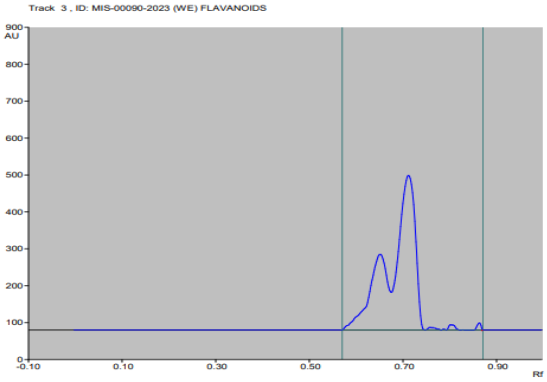
Graph 14: Spectra of Kaempferol showing concentration in WE, EAE, BE



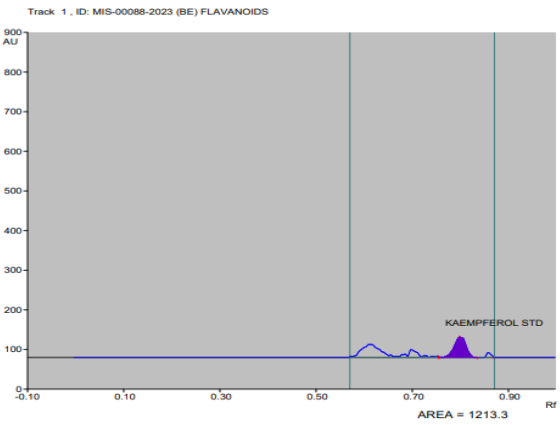
Graph 15: 3D View of Kaempferol showing concentration in WE, EAE, BE



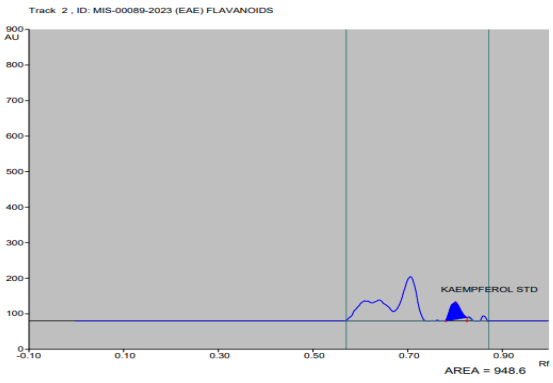
Graph 16 : Standard Kaempferol



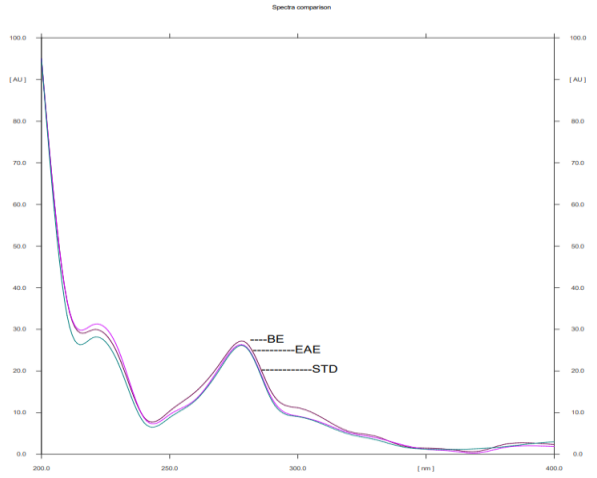
Graph 17 : Kaempferol in Water Extract (WE)



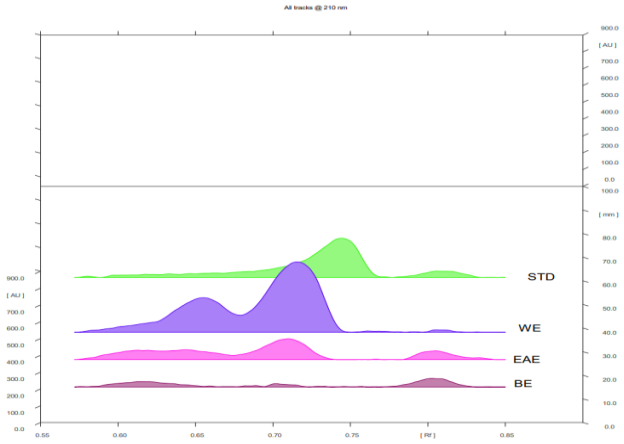
Graph 18 : Kaempferol in Butanol Extract (BE)



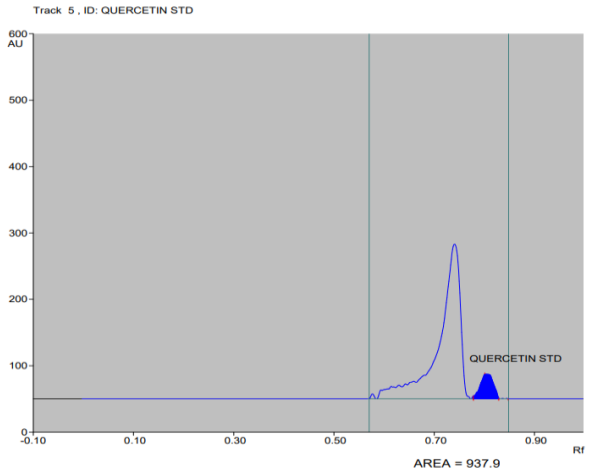
Graph 19 : Kaempferol in Ethyl acetate Extract (EAE)



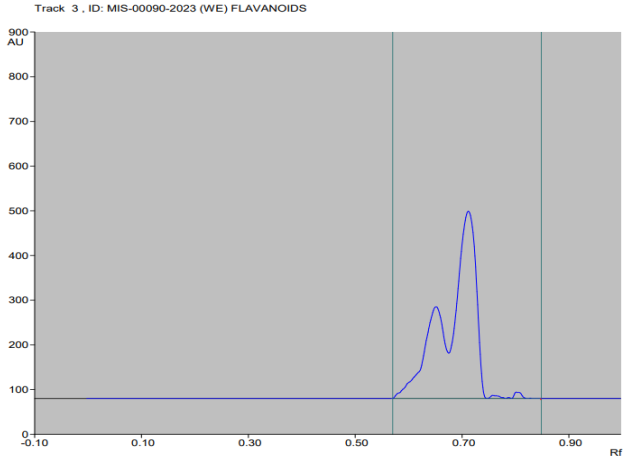
Graph 20: Spectra of Quercetin showing concentration in WE, EAE, BE



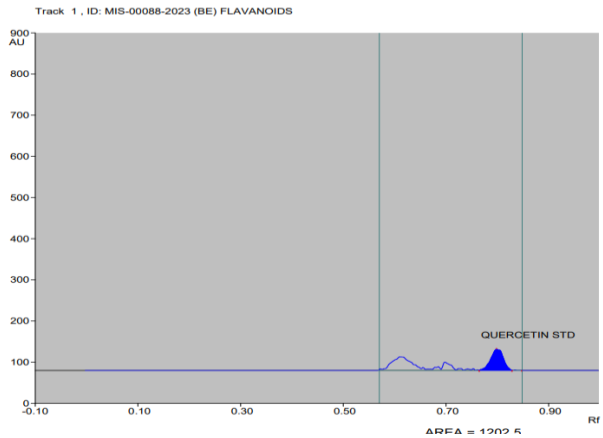
Graph 21 : 3D View of Quercetin showing concentration in WE, EAE, BE



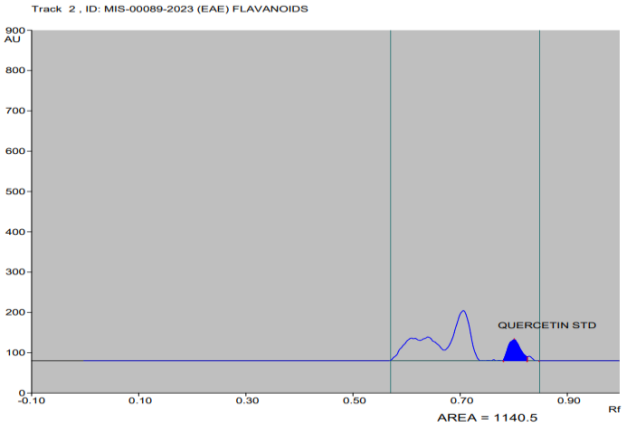
Graph 22: Standard Quercetin



Graph 23: Quercetin in Water Extract (WE)



Graph 24: Quercetin in Butanol Extract (BE)



Graph 25: Quercetin in Ethyl acetat Extract (EAE)

extract as many flavanoids as possible, the extract was fractionated with water, ethyl acetate, and butanol. Each fraction is put through an HPTLC analysis to measure specific flavanoids such as catechin, quercetin, taxifolin, and kaempferol. According to the findings, the butanol and water fractions did not include all of the four flavanoids i.e. some flavanois missing in water fraction and some missing in butanol fraction , whereas the ethyl acetate fraction did include all four with remarkable quantity The presence of flavanoid chemicals in the ethyl acetate fraction may be the primary cause of a variety of pharmacological effects, supporting the use of these molecules in therapies.

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