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Phytochemical Studies and GC-MS Analysis of *Tinospora Formanii* Leaf Extracts

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Abstract: ***The present study deals with the phytochemical aspects of Tinospora formanii. Crude leaf extracts were prepared with different solvents such as hexane, chloroform, methanol, and water using a Soxhlet apparatus. Qualitative and quantitative phytochemical studies and GC-MS analysis of leaf extracts have been carried out. Phytochemical studies showed that methanol leaf extract showed the presence of alkaloids, flavonoids, phenols, sterols, phytosterols, terpenoids, Tannins, saponins, glycosides, carbohydrates Anthraquinones, coumarins, Amino acids. Quantitative estimation showed that the methanol leaf extract exhibited a high flavonoid (74.25±0.885), phenol (57.22±0.135), and alkaloid (49.11±0.356) content. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified compounds such as 4-Hydroxy-3-methoxybenzyl alcohol, Humulen-[V1], Tridecene, Flavone, 9-hexadecenoic acid, Heptadecanoic acid, 1-Tricosene. The recorded phytocompounds are identified as bioactive constituents. The isolation of these bioactive compounds from T. formanii methanol leaf extract and their further studies in vivo animal models may lead to the development of new drugs.***

Keywords: Tinospora formanii, leaf extracts, **GC-MS analysis**

I. INTRODUCTION

The Indian healthcare scenario has inherited a large number of traditional practices, systems, and medicines as part of its holistic healthcare system, some of them more than 3000 years old. Herbal remedies are considered the oldest forms of health care known to mankind on this earth (Rahamtulla et al., 2020). India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of society either directly as folk remedies or indirectly as pharmaceutical preparations of modern medicine. Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al 2005).

Since herbal medicines are prepared from materials of plant origin, they are prone to contamination, deterioration, and, variation in composition. A lot of analytical techniques have been developed for quality control of drugs from plant origin. Therefore, it is very important to undertake phytochemical investigations along with biological screening to understand the therapeutic dynamics of medicinal plants and also to develop quality parameters. Plants and plant-based drugs are relatively less toxic and have acceptable side effects.

It is therefore essential to bring the use of the remedies into an existing framework or rational scientific use of medicines. A valuable approach is employing techniques like pharmacognostic and phytochemical studies, contributing to plant identity standardization. Despite the vast availability of medicinal plants, *Tinospora formanii* plant leaves are selected in our study and the case for the collection of material and its unique importance in this area.

II. MATERIALS AND METHODS

A. Sample Collection and Preparation of Solvent Extracts of T. formanii

T. formanii leaves were collected from the herbal garden of Acharya Nagarjuna University (Guntur, India) and shade-dried. Subsequently, the leaf material was carefully removed from polythene bags, washed with tap water, fragmented into small pieces, and subjected to shade drying for 35-40 days (Rahamtulla et al., 2023). Following the shade-drying process, the plant material was transformed into a powdered form using an electrical grinder. The resulting powder was sieved and subsequently stored within air-tight glass containers. The dried leaf powder was extracted progressively (Wiart et al. 2004) using various solvents based on their polarities, such as hexane, chloroform, methanol, and water, using a Soxhlet apparatus (Lin et al. 1999). The extracts are concentrated and solvent-free at decreased pressure using a rotary evaporator. The dried crude concentrated extracts were weighed and kept in an airtight bottle until utilized for analysis to determine the extractive yield.

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B. Qualitative Phytochemical Screening of Leaf Crude Extracts

Qualitative phytochemical screening of leaf crude extracts involved analyzing primary metabolites (carbohydrates and proteins) and secondary metabolites (alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids). Standard protocols outlined by Harborne (1973) and Trease and Evans (1989, 1996) were followed for the

1) Test for Alkaloids

Individually, the Extracts were dissolved in dilute Hydrochloric acid and the resulting solution was filtered.

a) *Wagner's test (Iodine in Potassium Iodide):* A few drops of Wagner's reagent were added to the filtrate. The presence of alkaloids is shown by the formation of a reddish-brown precipitate.
b) *Mayer's test (Potassium Mercuric Iodine solution):* A few drops of Mayer's reagent were added to the filtrate. The presence of alkaloids is shown by the development of a creamy white precipitate.
c) *Dragendorff's Reagent (Potassium Bismuth Iodide):* A few drops of Dragendorff's reagent were added to the Filtrate sample. The presence of alkaloids is shown by the formation of a reddish-brown precipitate.
d) *Hager's Test:* A few drops of Hager's reagent were added to the filtrate. Alkaloids are detected by the formation of a yellow precipitate.

2) Test for Flavonoids

a) *Shinoda Test:* A few fragments of magnesium ribbon and a few drops of concentrated hydrochloric acid were added to the extract solution. Flavonoids are present as the color changes from red to pink after a few minutes.
b) *Ferric Chloride Test:* A small quantity of extract was treated with a few drops of neutral ferric chloride solution. The phenolic nucleus is indicated by the development of a blackish-green color.
c) *Lead Acetate Test:* A few drops of aqueous simple lead acetate solution were applied to the extract for the lead acetate examination. Flavonoids can be detected by the formation of a yellow precipitate.
d) *Hydrochloric Acid-zinc Reduction Test:* A pinch of Zinc dust and a few drops of concentrated hydrochloric acid were added to the extract solution. Flavonoids are present when a magenta color develops after a few minutes.
e) *NaOH test/alkaline Reagent Test:* Apply a few drops of sodium hydroxide solution to the extract solutions. The presence of flavonoids can be seen by the intense yellow color that disappeared after adding dilute Hcl.

3) Test for Phenols

Ferric Chloride Test: Three drops of freshly prepared 1 percent Ferric chloride and Potassium Ferrocyanide were added to the filtered extract solution. The formation of a bluish-green colour is taken as positive.

4) Test for Sterols

a) *Salkowski Test:* A few drops of strong sulphuric acid were added to the extract solution and left to stand for a few minutes; the presence of red in the lower layer indicates the presence of sterols in the extract solution.
b) *Liebermann-Burchard Test:* To extract solutions, a few drops of acetic anhydride were added and thoroughly mixed. 1 ml of concentrated sulphuric acid was introduced through the test tube's sides. The presence of the reddish-brown ring suggests the presence of sterols Kokate (1994).

5) Test for Tannins

a) *Ferric chloride Test:* A few drops of 1% neutral ferric chloride solution were added to extracts, and a blackish-blue color was formed, indicating the presence of tannins.
b) *Gelatin Test:* 1 percent gelatin solution with 10% sodium chloride was added to the extracts. Tannins were detected by the formation of a white precipitate.

6) Test for Saponins

a) *Foam Test:* Shake a small amount of extract with a small amount of water for 10 minutes to see whether foam forms. It shows that saponins are present.

b) *Haemolysis Test:* 2 mL sterile water and 2 mL 1 percent sample extract were added to 2 mL 1.8 percent sodium chloride solution in two test tubes. 5 drops of blood were added to each test tube and the contents were gently mixed. The presence of saponins in the extract was determinedby haemolysis on a glass slide viewed under the microscope.
c) *Froth Test:* Added a drop of sodium bicarbonate solution to 5 mL of the plant extract. After 3minutes of intense shaking, the mixture was set aside. A honeycomb-like froth is formed.

7) *Test for Phytosterols*
a) *Liberman Burchard's test for Phytosterols:* Extract in a sterile test tube treated with chloroform and a few drops of glacial acetic acid and concentrated sulphuric acid at the side of the test tube. The red colour at the junction of the two layers and the upper layer shows a green colour.
b) *Salkowski Test:* Extract colored red or violet after being treated with equal parts chloroform and sulphuric acid.

8) *Test for Anthraquinones*
a) *Free Anthraquinones test (Borntrager's test):* The plant extract (equal to 100 mg) was vigorously agitated with 10 ml Benzene, filtered, and the filtrate was treated with 5 ml of 10% Ammonia solution. After shaking, a pink, red, or violet color in the ammonia (lower) phase suggests the existence of free anthraquinones.
b) *Detection of Coumarins:* The extract was treated with three (3) ml of 10% NaOH. The formation of yellow colour indicates the presence of coumarins.

9) *Test for Anthocyanins*
a) *Ammonia-HCl Test:* The extract was mixed with 2 mL of 2 N HCl and Ammonia to determine the presence of anthocyanins. The presence of anthocyanins is shown by the initial appearance ofapink-red color changing to blue-violet.

10) *Test for Terpenoids*
a) *Chloroform - H2S04 Test:* A volume of 5 ml of plant extract was combined with 2 ml of chloroform and a layer of concentrated H2S04 was added. The interface developed a reddish-brown coloration, indicating the presence of terpenoids.

11) *Test for Amino acid/ Protein*
a) *Ninhydrin Test:* In a boiling water bath, heat 3 mL of extract and 3 drops of Ninhydrin solution for 10 minutes. The presence of amino acids is indicated by the appearance of a purple color.
b) *Biuret Test:* Added 4 percent Na0H and a few drops of 1% copper sulfate solution to 3 ml of extract. Protein is confirmed by the formation of a violet color.
c) *Millon's reagent Test:* Mixed the extract with Millon's reagent in a test tube. Protein can be detected by the formation of a brick-red precipitate.
d) *Xanthoproteic Test:* concentrated nitric acid was added to one ml of extract, heated for one minute, and then liquid ammonia was added. The precipitate was formed.

12) *Test for Glycosides*
a) *Legal Test:* After dissolving the extract in pyridine, sodium nitroprusside and sodium hydroxide were added. Glycosides are identified by their deep red color.
b) *Baljet Test:* Sodium picrate solution was added to the extract in the Baljet test. The formation of a yellow color indicates the presence of glycosides.

13) *Test for Carbohydrates*
A small amount of extracts/fractions were diluted in a small amount of distilled water and then filtered separately to remove any impurities. The presence of carbohydrates in the filtrates was determined by testing the filtrates.
a) *Molisch's Test:* The extract was treated with Molish reagent, and concentrated sulphuric acid was applied to the test tube from the sidewalls of the test tube after that. The presence of carbohydrates is indicated by the appearance of a reddish-violet ring.
b) *Fehling's Test:* HydrolyZed filtrates were neutraliZed with alkali and boiled in equal parts Fehling's A and B solutions. The production of a green-to-yellow-to-red precipitate revealed the presence of reducing carbohydrates.

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14) Test for Fixed oils and fats

A drop of condensed extracts was squeezed between two filter papers and left undisturbed to test for fixed oils and fats. The presence of oil and fats is indicated by oil strains on the paper.

C. Quantitative Estimation of Phyto Constituents

The magnitude pertains to the inherent worth of the drug, signifying the concentration of medicinal components within. The existence of diverse phytoconstituents influenced the biological efficacy of the plant. Phenols, flavonoids, alkaloids, and other phytoconstituents were acknowledged to interact synergistically, necessitating their quantification in the plant extract.

D. Analysis of total Phenolic Content

A quantitative assessment of total phenolics was performed using the Folin-Ciocalteu reagent method, as outlined by Maurya and Singh (2010). Gallic acid served as the standard, and methanol solutions with concentrations ranging from 0.02 to 0.10 mg/ml were prepared, alongside a 1 mg/ml plant extract in methanol. Samples of 0.5 ml were mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent and 2 ml of 7.5 percent sodium carbonate in test tubes. After 30 minutes at room temperature, resulting in a blue color change, spectrophotometric absorbance was measured at 760 nm. Utilizing a calibration curve, the total phenolic content was determined in triplicate for each extract and standard. Results were expressed as milligrams of gallic acid equivalent per gram of extracts.

E. Total flavonoid content (TFC) Determination

The total flavonoid content of the extracts was quantified using the aluminum-chloride colorimetric method by Biju et al. (2014). In 10 ml volumetric flasks, 1 ml of 1 mg/ml extract was mixed with 4 ml of distilled water. Subsequently, 0.30 ml of 5% sodium nitrite and 0.30 ml of 10% AlCl3.6H2O solution were added sequentially, followed by 2 ml of 1.0 M NaOH solution after 5 additional minutes. The solution was diluted to volume with distilled water, and absorbances at 510 nm were measured using a UV/visible spectrophotometer against a reagent blank. Extracts and standard quercetin solutions (0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml) were analyzed in triplicate. Total flavonoid content was determined using a calibration curve and expressed as milligrams of quercetin equivalent per gram of extract, following the method by Kostic et al. (2013).

F. Determination of total Alkaloid Content

To identify alkaloids, the plant extract (1 mg/ml) was mixed with dimethyl sulfoxide (DMSO) and 2 N HCl, and then filtered. The resulting solution was combined with phosphate buffer and bromocresol green solution in a separating funnel. After vigorous shaking with varying volumes of chloroform (1, 2, 3, and 4 mL), the collected mixture was transferred to a 10-mL volumetric flask and diluted with chloroform to the mark. Reference solutions of atropine (0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml) were prepared similarly. Using a UV/VIS spectrophotometer at 470 nm, the absorbance of the solutions, including a reagent blank, was measured according to the method established by Tambe and Bhambar in 2014. The calibration curve enabled the determination of total alkaloid content, expressed as milligrams of atropine equivalent per gram of extracts. Analysis was conducted in triplicate for both extracts and standards.

G. GC-MS analysis of methanol extracts was analyzed by Gas Chromatography

The methanol leaf extracts were subjected to GC-MS analysis using an HP-5 column (30 m X 0.25 mm, 0.25 mm film thickness; Agilent Technologies 6890 N JEOL GC Mate II GC-MS model). The chromatographic conditions included helium as the carrier gas at a flow rate of 1 mL/min, injector temperature at 200°C, and a column oven temperature program of 50-250°C at a 10°C/min injection rate. Mass spectrometry conditions consisted of an ionization voltage of 70 eV, ion source and interface temperatures of 250°C each, and a mass range of 50-600 mass units (Rahamtulla et al., 2023).

For the identification of photo components, GC-MS mass spectra were analyzed using the National Institute of Standards and Technology (NIST) database, featuring over 62,000 patterns. A comparison of the mass spectrum of unknown phytocomponents with known components in the NIST library was performed. Subsequently, the names, molecular masses, and structures of the phytocomponents from the leaf extracts were determined. Chemical compound structures were generated using ChemDraw software.

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III. RESULTS & DISCUSSION

A. Physicochemical Assessment of Tinospora formanii

In the present study, *T. formanii* extracts are analyzed for their physicochemical characteristics (Table 1). The extraction process involved using nonpolar to polar solvents. A noticeable color variation in the extracts indicated the presence of diverse compounds in the solvent extracts, signifying the heterogeneous nature of the plant's constituents. Additionally, the study revealed that bioactive compound dissolution increased as the solvent polarity shifted from nonpolar to polar (Fig. 1). This observation further supported the correlation between solvent polarity and the solubility of bioactive components (Sultana et al., 2009). These results offer valuable insights into the composition of *Tinospora formanii* extracts and their potential applications.

Table 1 Physicochemical characteristics of *T. formanii* leaf extracts

Solvent used	The initial weight of the powder (g)	The final weight of the powder (g)	Weight of the crude extract (g)	Crude extract %	Color of the extract
Hexane	856.20	826.20	30.00	3.50	Brown
Chloroform	967.10	943.10	24.00	2.48	Dark Grey
Methanol	843.50	756.60	86.90	10.30	Dark Green
Water	859.30	839.00	20.30	2.36	Dark Green

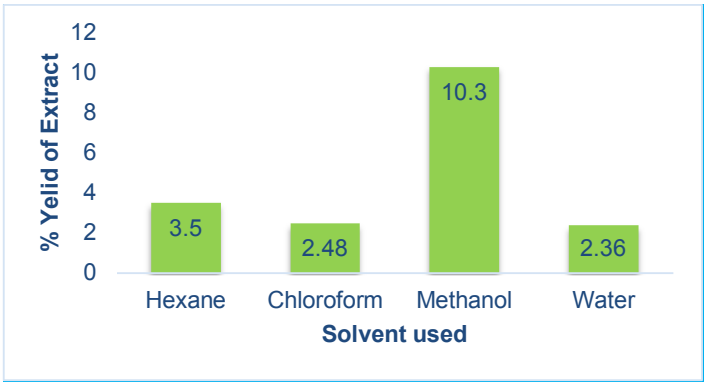


Fig. 1 Physicochemical characteristics of *T. formanii* leaf extracts

B. Phytochemical Studies

The initial qualitative analysis of *T. formanii* leaf extracts revealed the presence/absence of various metabolites, offering valuable insights into their chemical composition. This data served as a foundation for further exploration and potential applications of these extracts in diverse fields.

C. Qualitative Analysis

The qualitative analysis of the *T. formanii* leaf extracts showed the phytochemical composition of the various extracts (Table 2). The present study found that leaf extracts from *T. formanii* contain various phytochemicals, including alkaloids, flavonoids, phenols, sterols, phytosterols, and terpenoids. Tannins were present in all extracts, while saponins, glycosides, and carbohydrates were only found in methanol and water extracts. Anthraquinones were present in chloroform and methanol extracts of leaves, while coumarins were found in all extracts except chloroform. Amino acids were absent in hexane but present in other extracts. Oils and fats were observed in hexane and chloroform extracts. Anthocyanins were absent in all extracts. Methanol and water extracts showed more secondary metabolites, suggesting their better solubility in polar solvents.

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Table 2 Preliminary phytochemical analysis of *T. formanii* leaf extracts

S. No.	Tests	Hexane Extract	Chloroform extract	Methanol extract	Water extract
01	Test for Alkaloids				
	Wagner's test	+	+	+	+
	Mayer's test	+	+	+	+
	Dragendorff's test	+	+	+	+
	Hager's test	+	+	+	+
02	Test for Flavonoids				
	Shinoda test	+	+	+	+
	Ferric chloride test	+	+	+	+
	Lead acetate test	+	+	+	+
	Zinc-hydrochloric acid reduction test	+	+	+	+
	Alkaline reagent test	+	+	+	+
03	Test for Phenols				
	Ferric Chloride Test	+	+	+	+
04	Test for Sterols				
	Salkowski test	+	+	+	+
	Liebermann-Burchard test	+	+	+	+
05	Test for Tannins				
	Ferric chloride test	+	-	+	+
	Gelatin test	+	-	+	+
06	Test for Saponins				
	Foam test	+	-	+	+
	Haemolysis test	-	-	+	+
	Froth test	-	-	+	+
07	Test for Phytosterols				
	Liberman Burchard's test	+	+	+	+
	Salkowski test	+	+	+	+
08	Test for Anthraquinones				
	Free Anthraquinones test	-	+	+	-
09	Test for Coumarins				
	NaOH Test	+	-	+	+
10	Test for Anthocyanins				
	Ammonia-HCl test	-	-	-	-
11	Test for Terpenoids				
	Chloroform - H_2SO_4 test	+	+	+	+
12	Test for Amino acid/ Protein				
	Ninhydrin test	-	+	+	+
	Biuret test	-	+	+	+
	Millon's reagent test	-	+	+	+
	Xanthoproteic test	-	+	+	+
13	Test for Glycosides				
	Legal test	-	-	+	+
	Baljet test	-	-	+	+
14	Test for Carbohydrates				
	Molisch's test	-	-	+	+
	Fehling's test	-	-	+	+
15	Test for oils and fats				
	Filter paper test	+	+	-	-

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Plant analysis for bioactive compounds holds significance in determining medicinal value (Sasidharan et al., 2011). This investigation highlights substantial variations in compounds like alkaloids, flavonoids, phenols, sterols, tannins, saponins, phytosterols, anthraquinones, coumarins, anthocyanins, terpenoids, amino acids/proteins, glycosides, carbohydrates, oils, and fats, compared to prior studies. Environmental factors like temperature, altitude, and rainfall influence these variances (Kokate et al., 2004). The methanol leaf extract exhibits diverse phytochemicals, positioning it as a potential source of valuable drugs. These constituents could contribute to health benefits, given their crucial roles in well-being (Santhi and Sengottuvel, 2016).

D. Quantitative Estimation of Phytoconstituents

1) Total Flavonoid content of T. formanii leaf

Flavonoids, consumed by humans for around 4 million years, offer diverse biological properties that enhance health and reduce disease risks. This study quantified flavonoids in *T. formanii* extracts using the aluminum chloride method, using quercetin as a positive control. Among the extracts tested, the highest flavonoid content was in the methanol leaf extract () (Table 3; Fig. 2). This extract displayed significantly greater flavonoid concentration than the other extracts. Additionally, all extracts showed dose-dependent activity, where higher concentrations led to increased flavonoid levels. The water extract contained more flavonoids than chloroform and hexane extracts. These findings highlight *T. formanii* extracts, especially the methanol leaf extract, as abundant sources of flavonoids, potentially contributing to health benefits and disease prevention.

Table 3 Total Flavonoid Content of *T. formanii* leaf

Extract	Total Flavonoid content (mg/g)
Hexane extract	17.26±0.527
Chloroform extract	32.34±0.537
Methanol extract	74.25±0.885
Water extract	48.76±0.268

*Expressed as milligrams of quercetin equivalent (QE) per gram of extracts.
**Mean ±SD (n=3) is used to describe each value.

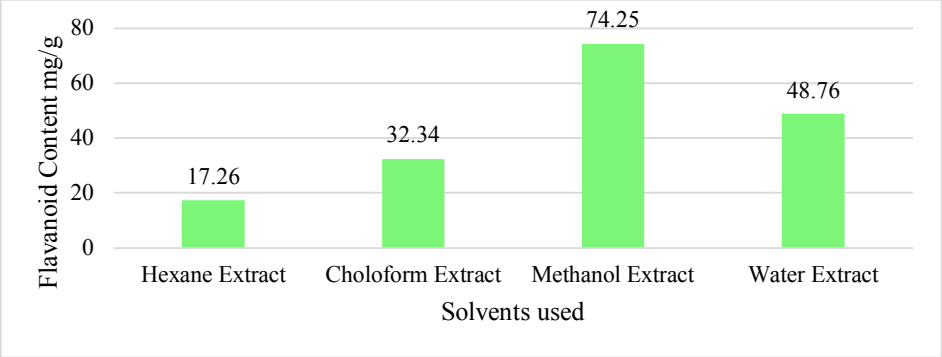


Fig. 2 Total Flavonoid content of *T. formanii* leaf

Flavonoids are important polyphenolic compounds abundantly present in plants and cannot be synthesized by the human body (McCullough et al., 2012). They exhibit a diverse range of biochemical and antioxidant properties, associated with various health conditions likecancer, Alzheimer's disease (AD), and atherosclerosis (Ovando et al., 2009; Lee et al., 2009). They play a crucial role in promoting health and find application in nutraceuticals, pharmacology, therapeutics, and cosmetics. Their powerful antioxidant, anti-inflammatory, antibacterial, anti-carcinogenic, and vascular activities, along with their influence on vital cellular enzymes, contribute to their health benefits. Additionally, they are potent inhibitors of various enzymes, including aldose reductase, calcium-dependent ATPase, xanthine oxidase (XO), cyclooxygenase(COX), lipoxygenase, and phosphoinositide 3-kinase (Metodiewa et al., 1997; Hayashiet al., 1998; Walker et al., 2000).

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2) Total Phenolic Content of T. formanii Leaf Extract

The total phenol content of the Total phenolic content of *T. formanii* Leaf extracts was determined by the Folin ciocalteu method where Gallic acid was used as a standard control (Karim et al., 2011). These results showed that the methanol extract of leaves possesses a higher number of phenols than other extracts (Table 4; Fig. 3). Hexane extracts contain less amount of phenol when compared to other solvent extracts, whereas methanol extracts contain a high quantity of phenol followed by water and chloroform extracts.

Table 4 Total phenolic content of *T. formanii* Leaf Extract

Extract	Total phenolic content (mg/g)
Hexane extract	10.70±0.214
Chloroform extract	19.86±0.117
Methanol extract	57.22±0.135
Water extract	43.73±0.758

* Expressed as milligram of gallic acid equivalent (GE) per gram of extracts
** Mean ±SD (n=3) is used to describe each value.

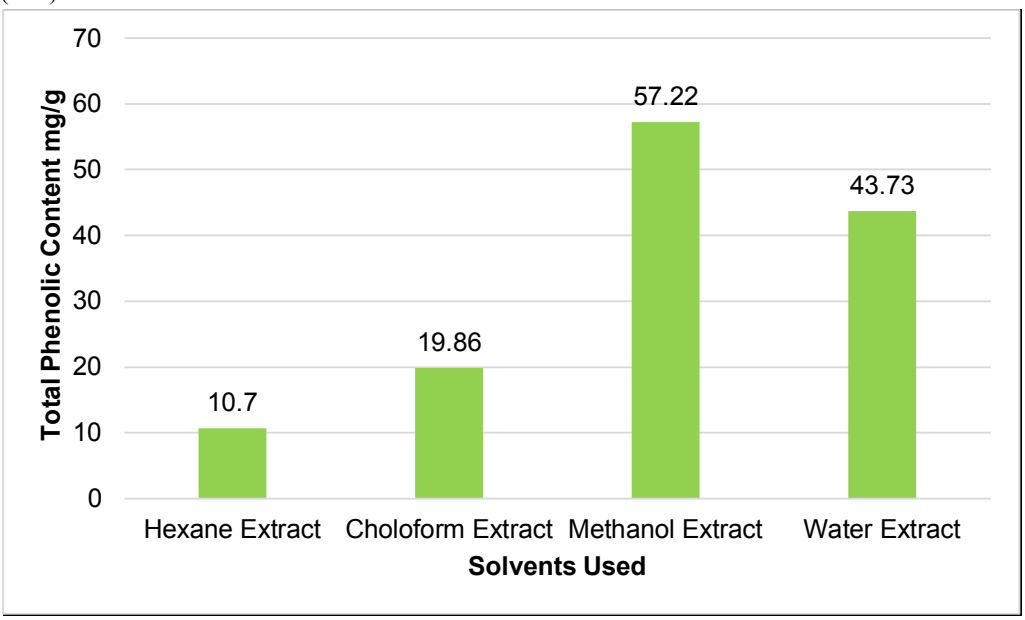


Fig. 3 Total Phenolic content of *T. formanii* Leaf

3) The total alkaloid content of T. formanii leaf extract

The present study revealed that alkaloids are abundant in methanol extract in comparison to the other three extracts (Table 5; Fig. 4). The extracts had dose-dependent activity, meaning that as the concentration was increased, the amount of alkaloids gradually increased. In comparison to chloroform and hexane extracts, water extract contained substantially more alkaloids.

Table 5 Total Alkaloid content of *T. formanii* leaf extract

Extract	Total Alkaloid content (mg/g)
Hexane extract	14.85±0.424
Chloroform extract	22.60±0.424
Methanol extract	49.11±0.356
Water extract	37.43±0.278

*Expressed as milligram of atropine equivalent CAE) per gram of extracts
** Mean ±SD Cn=3) is used to describe each value.

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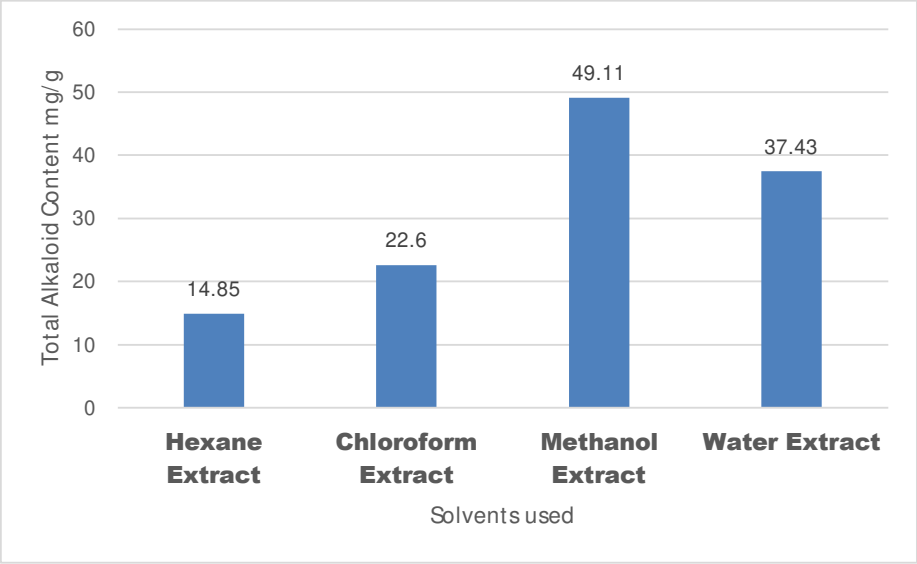


Fig. 4 Total Alkaloid content of *Tinospora formanii* Leaf Extract

Alkaloids represent a diverse group of over 6000 natural compounds, containing nitrogen and possessing pharmacological activity (Visweswari et al., 2013). They have various therapeutic applications and are sometimes used in tumor treatment. These compounds exhibit antimicrobial and sedative effects, acting as anesthetics to relax psychotic or hypertensive patients without inducing sleep. The health-promoting properties of alkaloids have been recognized for many years.These compounds demonstrate cytotoxicity and possess antimicrobial, antioxidant, antiprotozoal, antidiabetic, and anti-inflammatory properties. The alkaloids found in *T. formanii* extracts have shown potential health benefits, further highlighting the significance of these natural compounds in various therapeutic applications.

E. Gas Chromatography-Mass Spectrometry (GC-MS) analysis and Bioactive Compounds

The methanol leaf extract of *T. formanii* showed the highest number of phytoconstituents about 14 in its gas chromatogram (Fig. 5) and their structures, retention time, molar mass, and peak area percentage were shown in Table 6. Major phytoconstituents such as 4-Hydroxy-3-methoxybenzyl alcohol (Fig.6), 1,3-Pentadiene,2,4-di-t-butyl-, Humulen-[V1] (Fig.7), Tridecene (Fig.8), Phenol,2,4-bis[1,1-dimethylethyl]-, E-2-Tetradecen-1-ol, Flavone, 9-Hexadecenoic acid, Methyl ester, [Z], Hexadecanoic acid, methyl ester, 3, Octadecene, [E]-, 9-Octadecenoicacid[Z]-, methyl ester, Heptadecanoic acid, 16-methyl-, methyl ester, 1-Tricosene, Isopropyl stearate were noticed in the methanol leaf extract.

4-Hydroxy 3- methoxybenzyl alcohol (Vanillyl Alcohol) and 9-Hexadecanoic acid, methyl ester are recorded in *T. formanni* methanol leaf extract. These compounds possess anti-inflammatory, anti-angiogenic, and anti-nociceptive (Ahn et al., 2007; Lim et al., 2008; Jung et al., 2008

Flavone is recorded in the methanol leaf extract of *T. formanii*. Earlier reports indicate several biological activities such as Anti-inflammatory, antimicrobial (Cushnie & Lamb, 2005); anti-allergic, antioxidant (Havsteen, 1983); and antitumor activity (Harborne & Baxter, 1999; Verma & Pratap, 2010).

IV. CONCLUSION

The phytochemical studies of *T. formanii* conclude that the plant is therapeutically potent and supports the usage of leaves in folk medicine to treat various ailments. Further, this plant possesses several important bioactive compounds that are reported to have antibacterial, antifungal, antiviral, antioxidant, and anticancer properties. Further pharmacological investigation of *T. formanii* could produce many novel bioactive compounds.

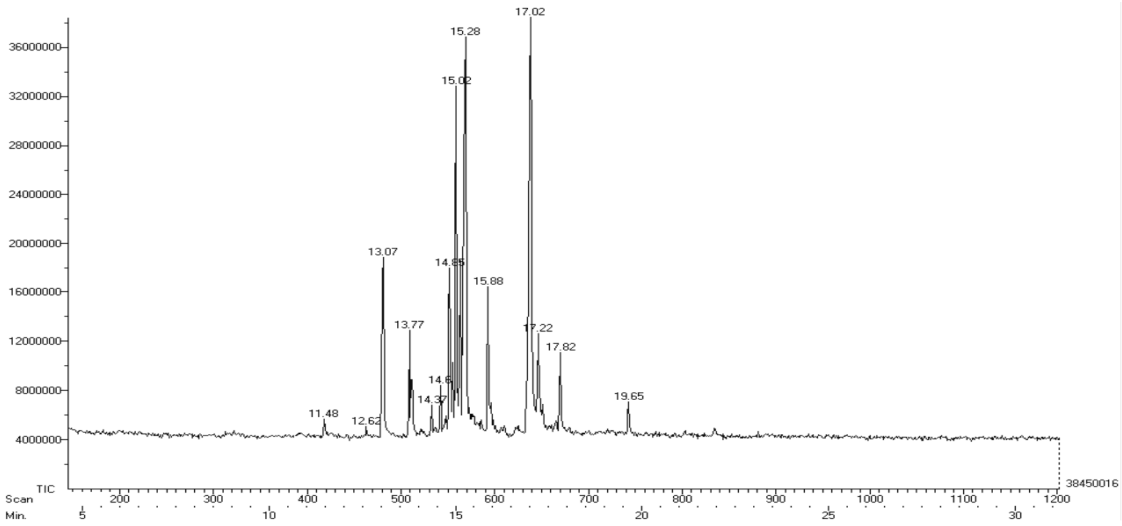


Fig. 5 GC-MS chromatogram of *T. formanii* methanolic leaf extract

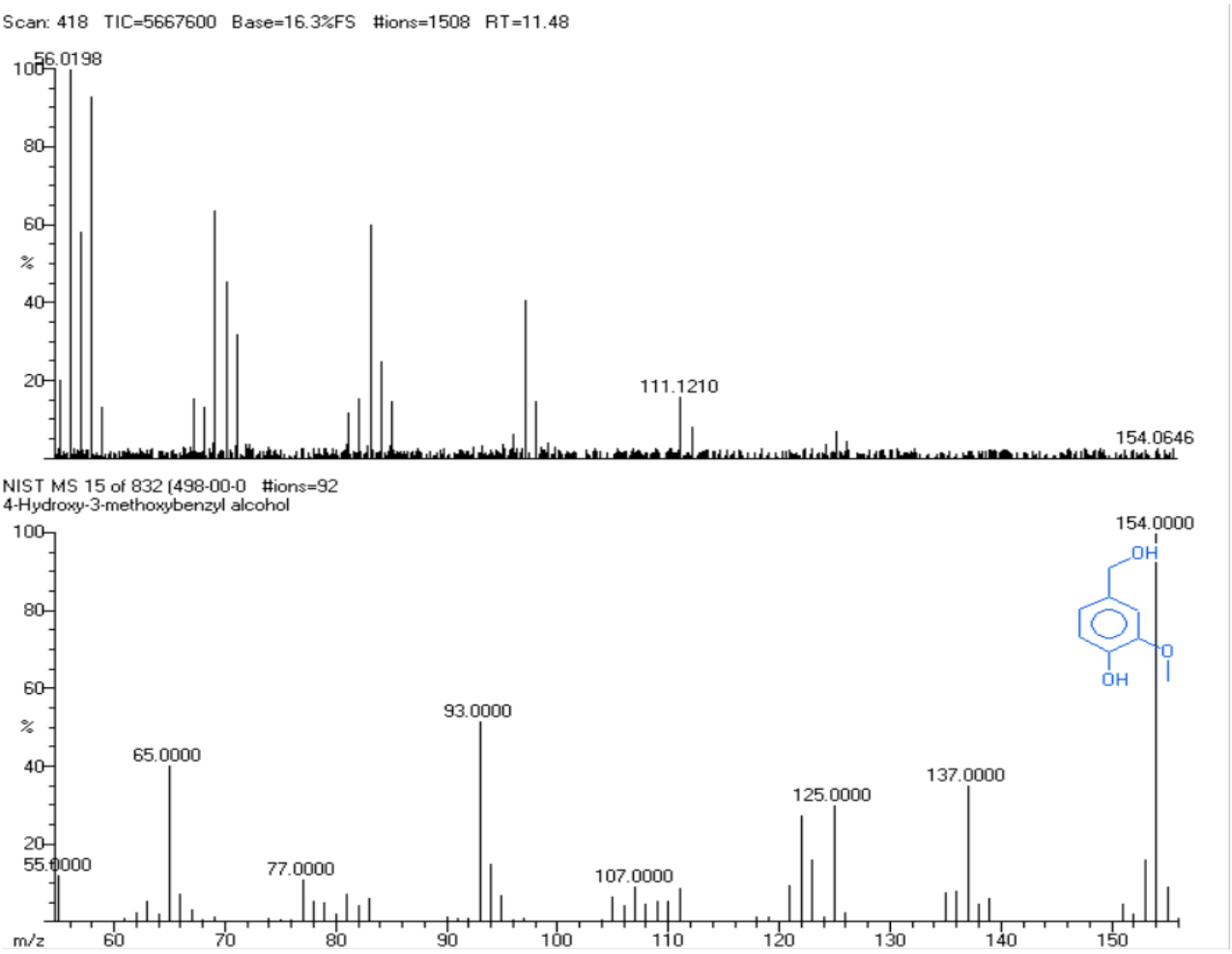


Fig. 6 Major phytochemical compound 4-Hydroxy3methoxybenzyl alcohol recorded in *T. formanii* methanolic leaf extract

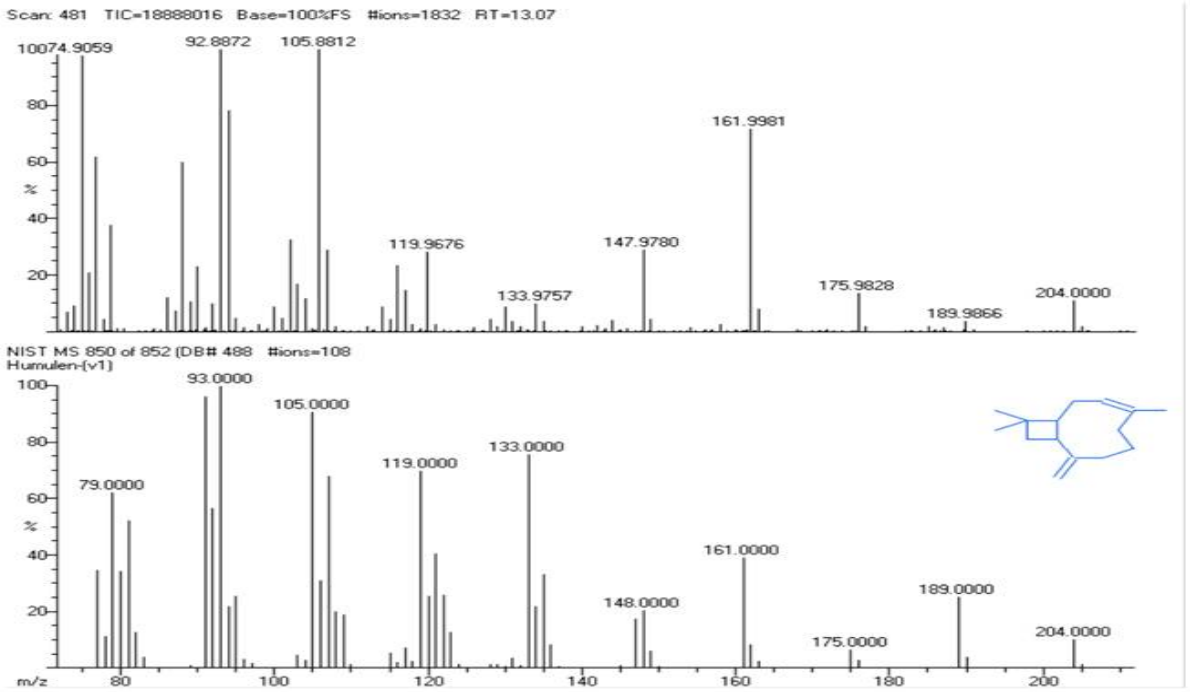


Fig. 7 Major phytochemical compound Humulen-[V1] recorded in *T. formanii* methanolic leaf extract

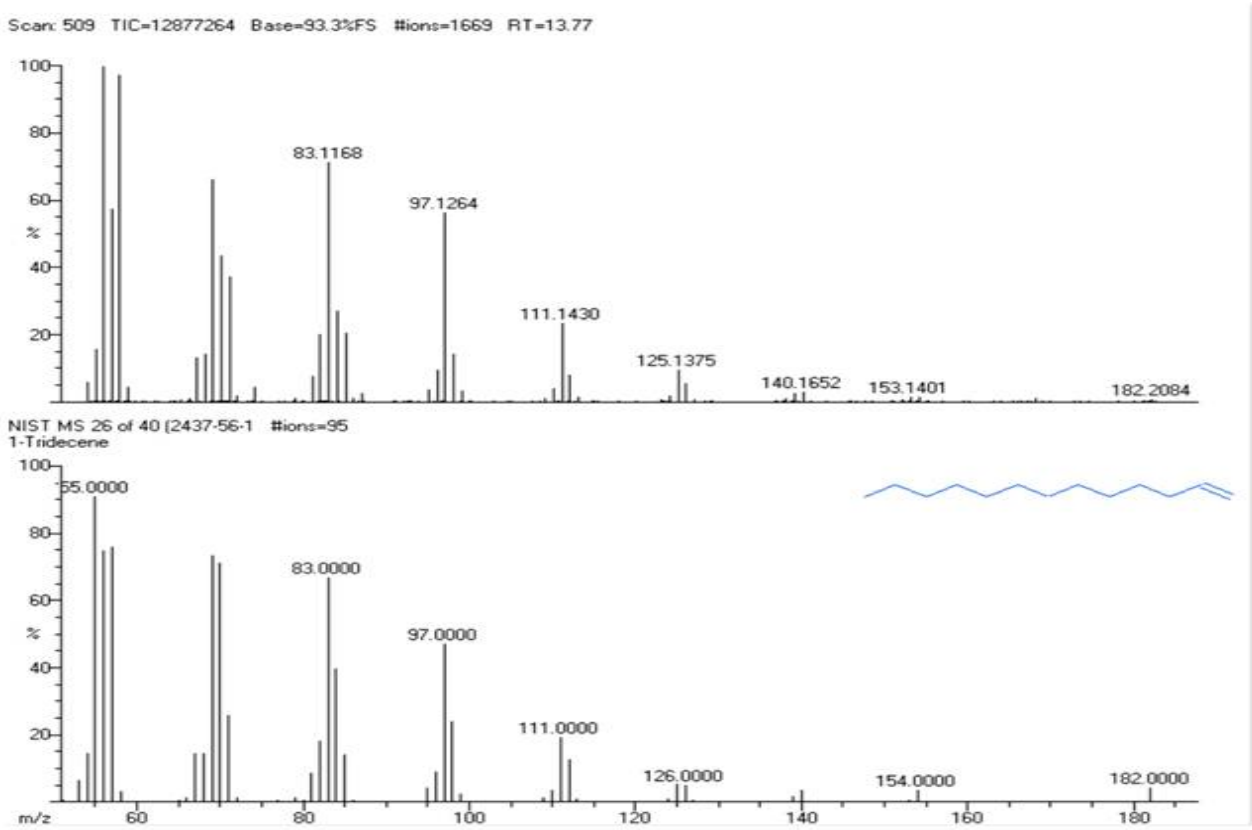
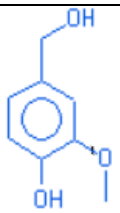
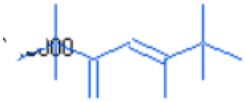
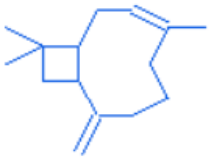
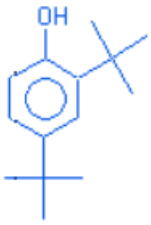
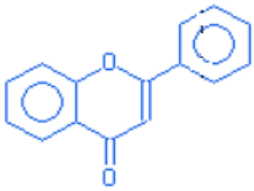


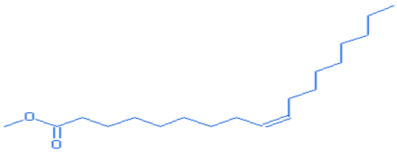
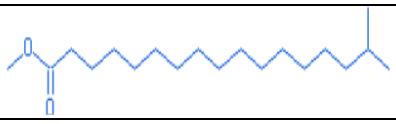
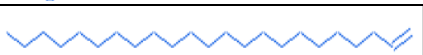
Fig. 8 Major phytochemical compound 1-Tridecene recorded in *T. formanii* methanolic leaf extract

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Table 6 Chemical compounds recorded from methanolic extract of *T. formanii*

S.NO	STRUCTURE	NAME OF THE COMPOUND	RT	MASS
1.		4-Hydroxy-3-methoxybenzyl alcohol	11.48	154.0000
2.		1,3-Pentadiene,2,4-di-t-butyl-	12.62	180.0000
3.		Humulen-[V1]	13.07	204.0000
4.		1-Tridecene	13.77	182.0000
5.		Phenol,2,4-bis[1,1-dimethylethyl]-	14.37	206.0000
6.		E-2-Tetradecen-1-ol	14.6	212.0000
7.		Flavone	14.85	222.0000
8.		9-Hexadecenoic acid, Methyl ester, [Z].	15.02	268.0000

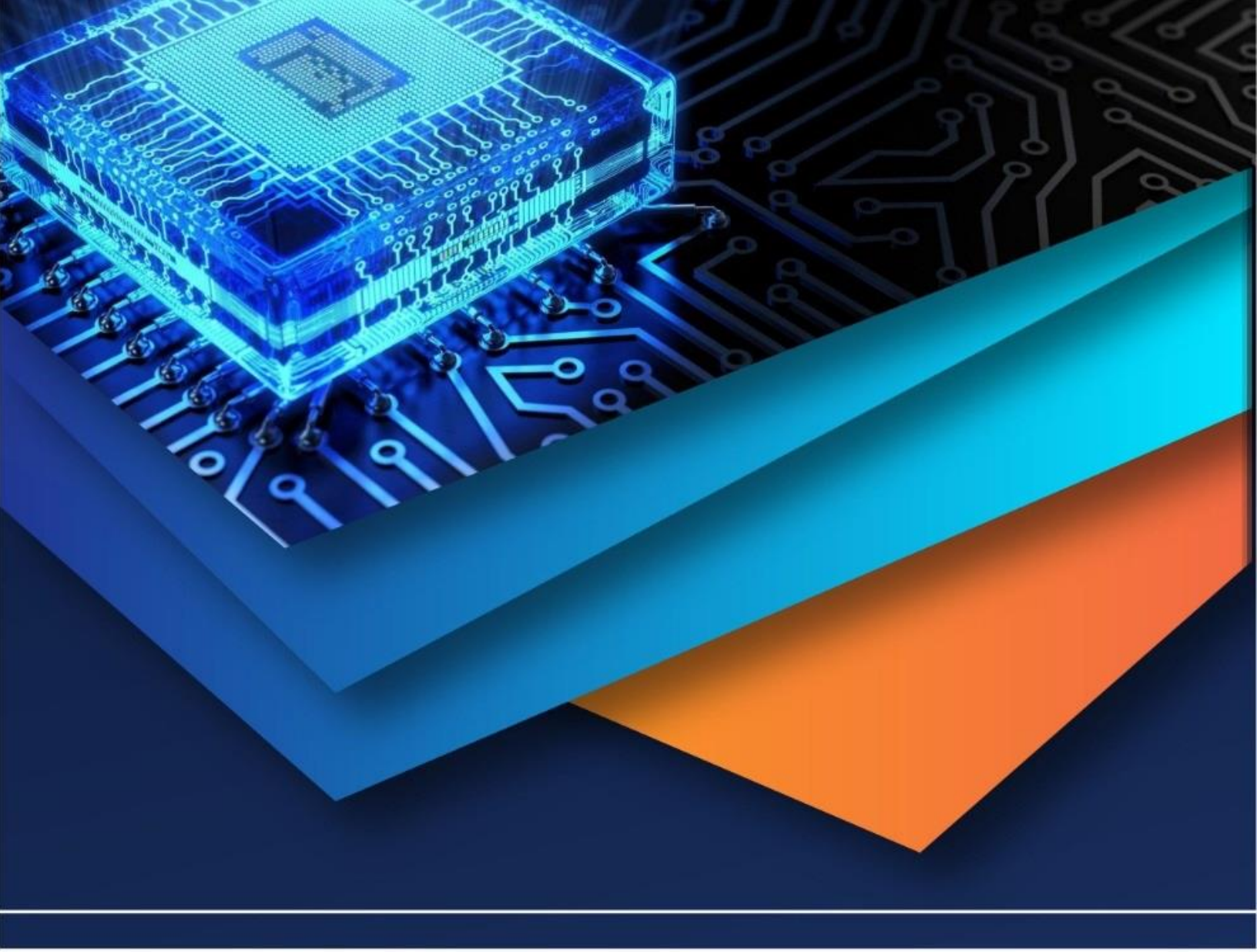
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9.		Hexadecanoic acid, methyl ester	15.28	270.0000
10.		3,Octadecene, [E]-	15.88	252.0000
11.		9-Octadecenoic acid[Z]-,methyl ester	17.02	296.0000
12.		Heptadecanoic acid, 16-methyl-, methyl ester	17.22	298.0000
13.		1-Tricosene	17.82	322.0000
14.		Isopropyl stearate	19.65	326.0000

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