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Phytochemical Estimation of *Chrysanthemum* flowers by FTIR Spectroscopy

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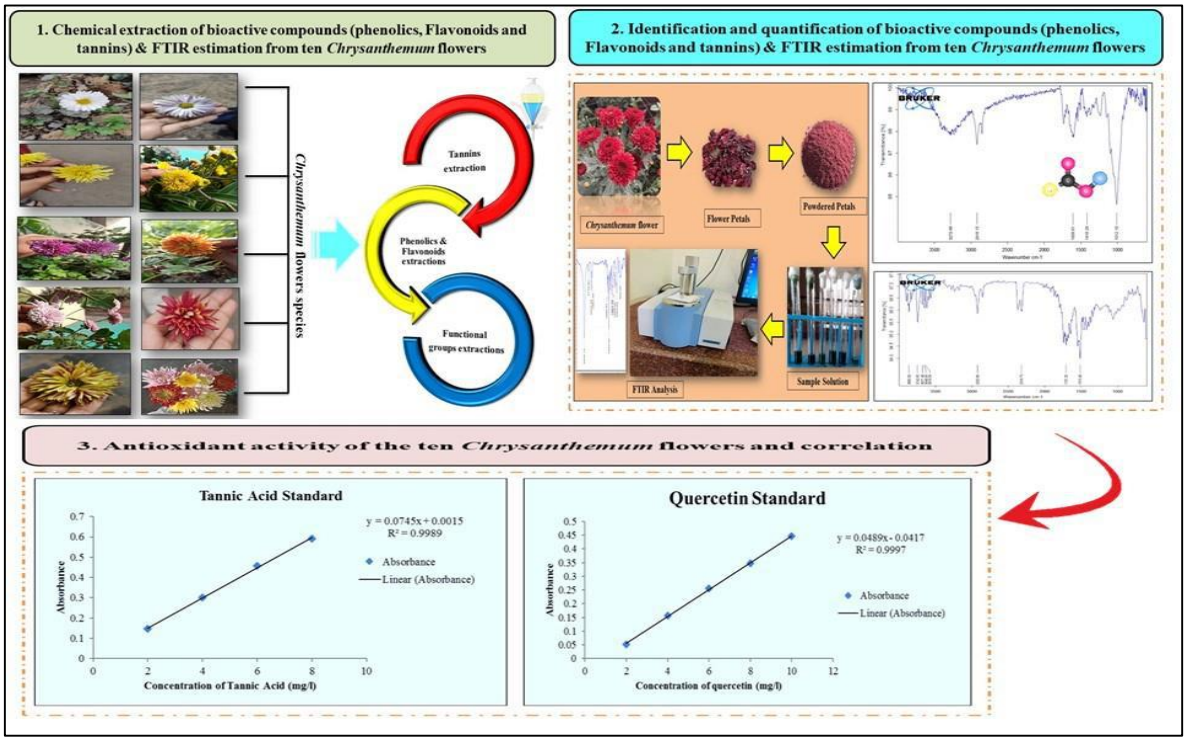
Abstract: *The purpose of this study was to assess the total phenol, tannin, alkaloid, and flavonoid contents in the Chrysanthemum flower extracts in petroleum ether, ethyl acetate, and methanol. Petroleum ether, ethyl acetate, and methanol were used as solvents in a continuous hot percolation method in a soxhlet apparatus to extract powdered wood material. For the Folin-Ciocalteu method of determining total phenol and tannin, gallic acid was utilised as the standard. Quercetin was used as a standard in the chloride colorimetric method to determine the total alkaloid content. Using atropine as a standard, the results demonstrated that ethyl acetate extract had a high concentration of total phenol, tannin, alkaloid, and flavonoid contents when compared to bromocresol green solution. Total flavonoid content was determined by aluminium to petroleum ether, ethyl acetate and methanol extracts. Ethyl acetate extract contained the total phenol of 30.18 and tannins of 83.03 as mg of gallic acid equivalents (GAE), alkaloids of 66.01 as mg of atropine equivalents (AE) and flavonoids of 91.01 as mg of quercetin equivalents (QE).*

Key Words: *Phenol, flavonoid; alkaloid; tannin;* Chrysanthemum *flower.*



Graphical Abstract

1. INTRODUCTION:

Plants have provided mankind with herbal remedies for several diseases for many centuries. In India herbal medicines have been the bases of treatment and cure for various diseases in traditional methods such as Ayurveda, Unani and Sidha. The therapeutic potentials of plant and animal origin crude drugs are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. Thus, the plant species may be consider as a biosynthetic and for the chemical compounds example proteins, carbohydrates, and fats that are utilized as food by the animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties.[1] In the current study, the petroleum ether, ethyl acetate, and methanol extracts of *Chrysanthemum* were examined to determine their total phenol, tannin, alkaloid, and flavonoid contents. Petroleum ether, ethyl acetate, and methanol were used as solvents in the continuous hot percolation process to extract the powdered wood material from the soxhlet device. The therapeutic potentials of plant and animal origin crude drugs are being used from the ancient times by the simple process without the isolation of the pure compounds. The pharmacological action of crude drug is determined by the nature of its constituents. Thus, the plant species may be considered as a biosynthetic and for the chemical compounds example proteins, carbohydrates, and fats that are utilized as food by the animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties.[1] *Chrysanthemum morifolium* is a species of perennial plant from the Asteraceae family. Also known as mums, *Chrysanthemum morifolium* is among the four most famous chrysanthemum species in China. According to traditional Chinese medicine principles, *Chrysanthemum morifolium* has been indicated to be 'cool' in nature and 'acrid' in taste, and exhibits affinity for the lung and liver systems (1). Furthermore, according to traditional Chinese medicine, chrysanthemum is able to improve eyesight and prevent fatigue (2). The main ingredients of *Chrysanthemum morifolium* are volatile oils (3), flavonoids (4), chlorogenic acid (5), polysaccharides (6), phenols (7) and trace elements (8). Flavonoid compounds, triterpenoids and volatile oils are the main active components (9). Flavonoids from *Chrysanthemum morifolium* may significantly improve the activity of antioxidases in the tissues of rats with lead poisoning, relieve lipid peroxidation and antagonize oxidative injury of the brain, liver and kidney (10).

2. CHEMICALS & REAGENT

Ferric Chloride, ethanol, distilled water, aqueous HCl, Acetic Anhydride, Lead Acetate, Ethanolic α Naphthol, Sodium hydroxide, Benzene, Chloroform, Concentrated Sulphuric acid, Ammonia solution, Folin- Ciocalteu reagent, Sodium Bicarbonate, Acetone, Potassium acetate and Aluminium Chloride hexahydrate.

SAMPLE COLLECTION

Ten *Chrysanthemum* flowers were collected locally from the various area in Raipur, Chhattisgarh. The plants collected were identified botanically in standard monographs **[16-26,7]**. Fresh flower petals are selected and further used for phytochemical analysis. Flowers species selected during present investigation are shown in **(Figure 1)**

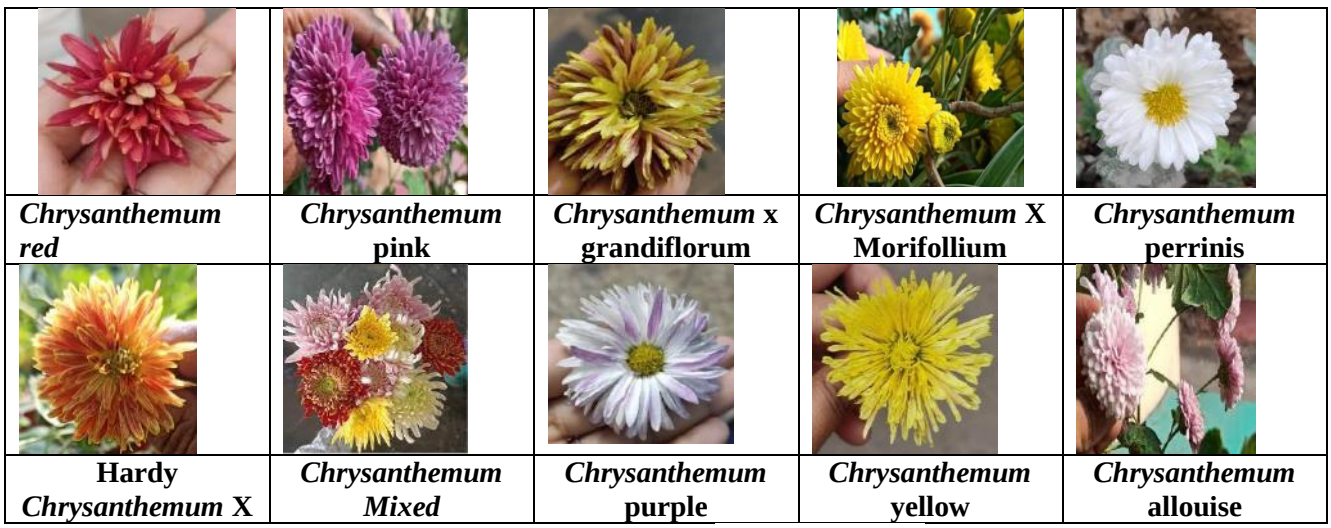


Figure 1: Representation of the *Chrysanthemum* flowers

PREPARATION OF PLANT EXTRACT

The leaves and flower petals of the selected plants were removed from the plants and then washed under running tap water to remove dust particles. The petals and leaves samples were then air dried for few days and the flower petals and leaves were crushed into fine powder and stored in polythene bags for use. The plant powder 0.1 g was taken in a test tube and 70% Acetone and Ethanol water was added to it for quantitative and qualitative tests respectively such that plant powder soaked in it and sonicate for extract preparation. The solution was then filtered with the help of Whatman filter paper and filtered extract of the selected flowers and leaves samples were taken and used for further phytochemical analysis.

3. QUALITATIVE PHYTOCHEMICAL ANALYSIS

The flower petals and leaves samples powder were dissolved in 70% Ethanol aqueous solution and then were assessed for the existence of the phytochemicals by using the following standard methods **[8-13]** as described in **[Table:1]**.

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content (TPC) of each extract was determined as tannic acid equivalents by using the Folin-Ciocalteu reagent. According to this method, aliquots were prepared by dissolving 0.1 g of extract in 70% acetone solution. The reaction mixture consists of 0.2 ml of the sample extract, added to a test tube containing 7.3 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, 2 ml of 20 % $NaHCO_3$ solution and make up to 10 ml of total volume. A set of reference standard solutions of tannic acid (2.0, 4.0, 6.0, and 8.0 mg/l) were prepared in the same manner as described **[14]**. Absorbance for test and standard solutions were measured against the blank at 735 nm with an UV/Visible spectrophotometer. Absorbance was determined in triplicate for all extracts. The tannin content was expressed in terms of Tannic acid mg/kg of extract, respectively.

DETERMINATION OF TOTAL FLAVONOID CONTENT

The flavonoid content was determined by the Aluminum Chloride method with quercetin as standard **[15]**. According to this method, aliquots were prepared by dissolving 0.1 g of sample in 70% acetone solution. 0.2 mL of sample solution was taken out and was mixed with 0.1mL of 1M potassium acetate (CH_3COOK), 0.1mL of 10% aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) and diluted with 4.6 ml distilled water to make up a total volume of 5ml. A set of reference standard solutions of Quercetin (2.0, 4.0, 6.0, 8.0 and10 mg/l) were prepared in the same manner as described **[16-17]**. The slope (20.5) and intercept (0.85) derived were employed for computing concentration of flavonoid in the sample solution. Absorbance for test and standard solutions were measured against the blank at 415 nm with an UV/Visible spectrophotometer. All determinations were carried out in triplicate. Total flavonoid content was expressed in terms of quercetin mg/kg of extract, respectively.

4. RESULTS & DISCUSSIONS :

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The presence of carbohydrates, proteins, coumarins, terpenoids, tannin, and phenols were found in the ethanolic extracts, whereas anthraquinones were not found. The purpose of this investigation was to determine the presence of secondary metabolites in the sample of (n=30) hibiscus flower petals and leaves. The sample plants' therapeutic qualities may be due to the existence of numerous bioactive secondary metabolite chemicals. Following a number of assays, the secondary metabolites and their phyto-constituents were identified and are shown in [**Table: 2,3**].

Table:1 Qualitative Phytochemical test results for n=30 flower samples.

S No.	Name	Tannin	Flavonoid	Terpenoid	Carbohydrate	Coumarin	Proteins	Anthraquinone
1.	*Chrysanthemum Pink*	+ +	+	+ ++	+	+ +	+	-
2.	*Chrysanthemum Yellow*	+ +	+ +	+	+ +	-	++	-
3.	*Chrysanthemum White*	+ +	-	+ +	+ +	+ + +	+ +	-

"+" = presence of compound, "+ + +" = highest concentration of the compound, "+ +" = higher concentration, "+" denotes high concentration of the compound, "-" = absence of the compound

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Total Tannin Content was estimated using spectrophotometry using the liner regression curve (y = 0.0744x + 0.0015, with a coefficient value of R^2 = 0.998) plotted against different tannic acid concentrations. The result was expressed as mg of tannic acid equivalents (TAE)/mg of dried extracts. The calibration curve standard depicted in [Figure: 2]. The concentration of tannin content in the sample solution was calculated using the slope (13.4) and intercept (0) that were found. Chrysanthemum Sherwood's Peach flower petals had the highest tannin concentration (67400 mg/kg) among the ten flower petals examined, while Chrysanthemum (Pale-Purple) flower petals had the lowest concentration (30490 mg/kg).

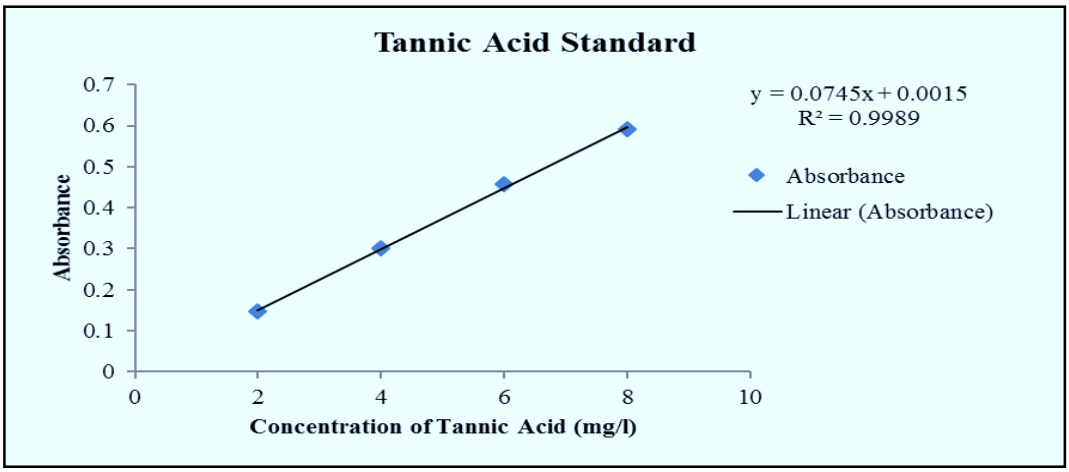


Figure 2 Calibration Curve for Tannic acid for determining the Tannin Content.

The total flavonoid content of (n=10) *Chrysanthemum* flower petals and leaves were determined using quercetin standard from their liner regression curve (y = 0.0489x - 0.0417 with coefficient of R^2 = 0.999) plotted with various concentrations quercetin and termed as mg of quercetin equivalents (QE)/mg of dried extracts. The standard calibration is curve shown in **[Figure: 3]**. The slope (20) and intercept (1) obtained was used for computing the concentration of TFC in the sample solution. The maximum amount of flavonoid and tannin are observed in flower petals of *Chrysanthemum* (Chrysanthemum X Morifollium) (145800 mg/kg) and *Chrysanthemum (Common* daisy) (66530 mg/kg) respectively and minimum amount is observed in flowers petals of *Chrysanthemum* (Pinkish white) (18000mg/kg) and *Chrysanthemum* (Pale-Purple Dinnerplate) (30390 mg/kg) respectively as shown **[Table 2]**. The findings of the present investigation explored the presence of broad range of flavonoid content with high significance in flower petals of *Chrysanthemum (Red)* species. The total concentration was found to be *Chrysanthemum* (Chrysanthemum X Morifollium) (145800mg/Kg) in flowers shows highest flavonoid concentration and *Chrysanthemum* (Pinkish white)(18000mg/Kg) in flowers shows lowest flavonoid concentration. The average flavonoid concentration found in flowers was 66190 mg/Kg. It is expected that the important phytochemical properties in the *Chrysanthemum* plants recognized by this study will enhance the interest of the people in its therapeutic value besides its decorative purposes. Figure 4

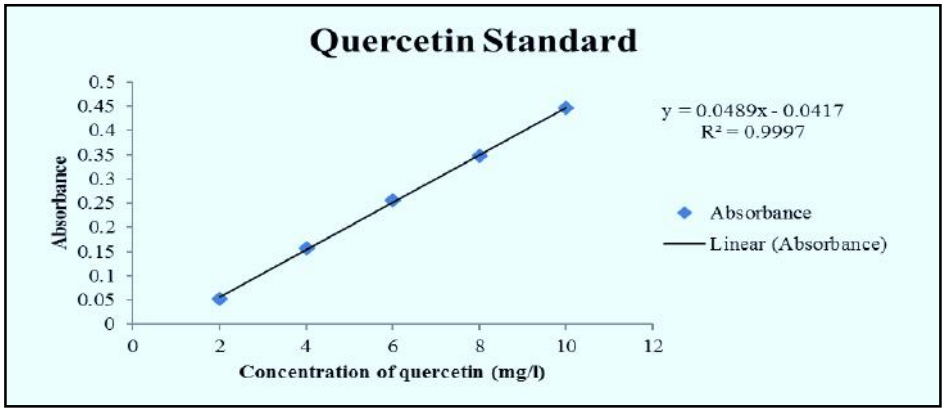


Figure 3 Calibration Curve for Quercetin for determining the Total Flavonoid content

Table 2: Data representation of the various flower petals in the *Chrysanthemum flowers*				
			Flower petals	
S.No.	**Botanical Name**	**Family**	**Tannin (mg/kg)**	**Flavonoid (mg/kg)**
1	*Chrysanthemum* (Bellis perennis)	Asteraceae	68400	103800
2	*Chrysanthemum* (Common daisy)	Asteraceae	66530	74200
3	*Chrysanthemum* (Chrysanthemum x grandiflorum)	Asteraceae	47300	102900
4	*Chrysanthemum* (White)	Asteraceae	53200	34100
5	*Chrysanthemum* (Pale-Purple Dinnerplate)	Asteraceae	30390	53700
6	*Chrysanthemum* (Chrysanthemum X Morifollium)	Asteraceae	58120	145800
7	*Chrysanthemum* (Hardy chrysanthemum)	Asteraceae	33200	42000
8	*Chrysanthemum* (Happy Purple)	Asteraceae	57120	32800
9	*Chrysanthemum* (Pinkish White)	Asteraceae	46160	18000
10	*Chrysanthemum* (Mixed)	Asteraceae	52870	54600
		Mini	**30390**	**18000**
		Max	**66530**	**145800**
		Mean	**51369**	**66800**

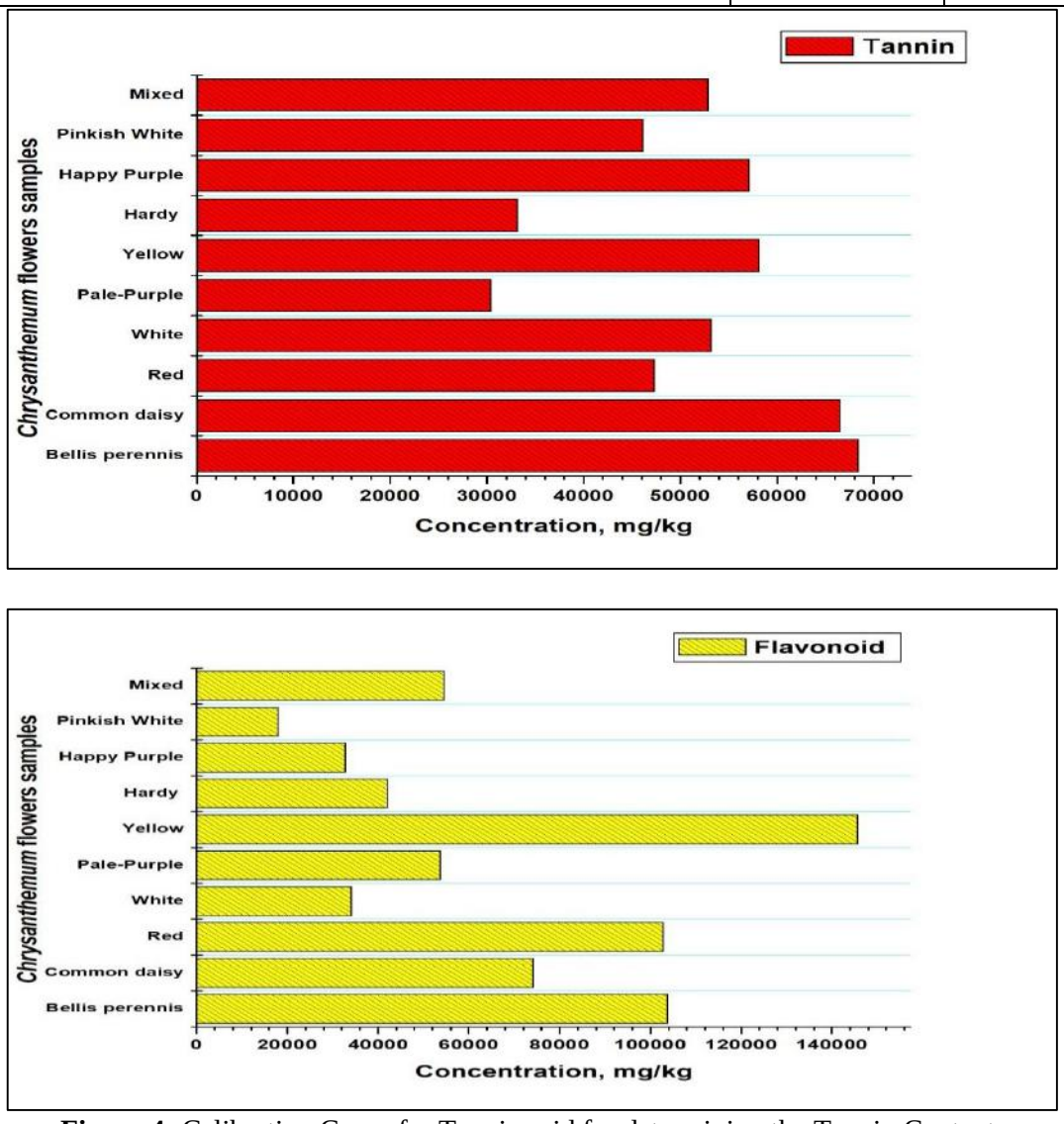


Figure 4: Calibration Curve for Tannic acid for determining the Tannin Content.

5. DISCUSSION:

Several thousand distinct compounds have been identified as members of the most prevalent class of secondary metabolites found in plants, known as polyphenols [18–20]. In plant biology and human life, polyphenols have a wide range of functions, such as serving as UV protectors, defence mechanisms against infections and herbivores, producers of plant colours, enhancers of food and drink flavour, and ingredients in pharmaceuticals [21–22]. Flavonoids are abundant and widely distributed in the kingdom of plants. Since many flavonoids are yellow in colour, the word "flavonoid" comes from the Latin word flavus, which means yellow. The unique flavonoid-related anthocyanins, on the other hand, are red, blue, or purple in many others, who are white. Additionally, flower petals and leaves contain flavonoids [**23–26**].

FTIR ESTIMATION

Herbs used for healing are an essential source of phytochemicals that provide traditional medical care for various ailments [12]. The primary source of functional components used to enhance novel chemotherapeutic agents is plants [13]. Vibration spectroscopy, or Fourier transform infrared (FTIR) spectroscopy, uses infrared radiation to cause molecular bonds in the sample it absorbs to vibrate. Since the majority of examples have different subatomic bonds or unique subatomic bond configurations, FTIR makes it possible to obtain compound data on the particles within the specimen [14]. Based on the peak values in the region of IR radiation, the FTIR spectrum is used to identify the functional groups of the active ingredients present in the extract. The practical groups of the components are separated at the moment when the concentrate is fed into the FTIR, providing a clear picture of its peak proportion. The ethanolic extract of *Chrysanthemum* flowers contains a variety of chemical constituents, including alcohol, alkanes, aromatic carboxylic acid, halogen compounds, and alkyl halides, as confirmed by the results of FTIR spectroscopy. The ethanolic extract of *Chrysanthemum* flowers was found to contain 17 functional groups, as shown in Table 1 and Figure 1. The strong instance peaks are identified at 3348.42 and 1643.35 cm^{-1} which are assigned to the H-bonded and O-H stretching vibration The peaks at 2090.84,1990.54 and 1851.66 cm^{-1} which are assigned to the carbonyl compound frequency vibration. Based on the peak values in the region of IR radiation, the FTIR spectrum is used to identify the functional groups of the active ingredients present in the extract. The ethanolic extract of *Chrysanthemum* flowers was found to contain 17 functional groups, as shown in Table 1 and Figure 1. The results of FTIR analysis confirm the presence of functional groups such as non bonded, O-H stretch, carboxylic group, acidic, H bonded, C-H stretch, asymmetric stretching of –CH (CH_2) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO3 stretch, =C-H bending and C-Cl. Which shows the major peak values of 3942.50, 3880.78, 3765.05, 3726.47, 3317.56, 3248.13, 3055.24, 2924.09, 2854.65, 2661.77, 2569.18, 2422.59, 2353.16, 2314.58, 2175.70, 2036.83, 1982.82, 1936.53, 1851.66, 1712.79, 1651.07, 1442.75, 1381.03, 1327.03, 1265.30, 1195.87, 1087.85, 1049.28, 879.54, 802.39 and 709.80 cm^{-1}. respectively in the Figure 2 and Table-2. An intense band occurs at 1049.28 cm^{-1} corresponding to PO3 stretch vibration indicates the presence of phosphate ion in the flower extract of *Chrysanthemum* flowers. The alkanes compounds are present in the wax of numerous species. They care for the plant against water loss, and protect against bacteria, prevent the leaching of important minerals by the rain, harmful insects, and fungi [16].

6. CONCLUSION:

This study examined the total phenol, tannin, alkaloid, and flavonoid contents of *Chrysanthemum* flower extracts in petroleum ether, ethyl acetate, and methanol. Comparing ethanol acetate extract to petroleum ether and methanolic extract, the former revealed higher concentrations of phenol, alkaloid, flavonoid, and tannin. The pharmacological action of *Chrysanthemum* flower ethyl acetate extract is contingent upon the characteristics of these chemical compounds, which confer the intended therapeutic effects and specific physiological effects. The plants under examination here may produce useful pharmaceuticals. In order to obtain valuable medicinal and antioxidant agents, we thus suggest further hibiscus bioactive compound isolation, identification, purification, characterization, and structural clarification. This supports the claims regarding the therapeutic benefits of this plant as a curative agent as well as its traditional medicinal uses.

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