Green Extraction, Synthesis, Characterization and Antifungal Screening of Copper (II) and Iron (III) Complexes of used Herbal Tea (Chamomile)

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Abstract:- The aqueous extract of spent chamomile tea was used to synthesize and characterize Cu^{2+}, and Fe^{3+} complexes and the antifungal activities of the complexes determined. The physiochemical parameters such as melting point, colour, percentage yield and solubility test were studied. The major Phyto-constituents present were alkaloids, Flavonoids and Tannins, which may be responsible for pharmacological activities. The extract and complexes were scanned in the wavelength ranging from 200-700nm using a UV-Vis spectrophotometer. The characteristic peaks showed a bathochromic shift in Copper (II) chloride salt. The Cu^{2+} and Fe^{3+} chamomile complexes with a Hypochromic shift in iron (iii) chloride salt. FT-IR of the crude extract and complexes contain alcohols, phenols, alkanes, amines, aromatic amine, aliphatic amine and alkyl halide in the extract and complexes with the only aromatic amine in the extract and alkyl halide in the Fe^{3+}chamomile complex. XRF analysis determined the element content and their percentages, showing a significant change in both extract and complexes, with the Fe^{3+}chamomile complex having the highest content of 11.7202%. The result of XRD showed the crystalline size of the complexes using the Scherrer formula as $D= K\lambda/\beta 2\theta \cos \theta$. The crystalline size was calculated for Cu^{2+} and Fe^{3+} complexes with a value of 73.1nm and 37.1nm, respectively. This shows that the copper complex is more crystalline. The antifungal study of the crude extract and complexes was tested on Aspergillusflavus, Aspergillusniger, Fusarium sp. and Penicilliumspp. at different concentration (mm/mg) of 12.5, 25, 50, 100 and 200. The complexes were biologically active and showed enhanced antifungal activity compared to the free ligand. The copper complex showed a better antifungal potency compared to the Iron complex. Chamomile can potentially be used as an active ingredient in the production of antifungal drugs.

***Keywords**:- Copper complex, zinc complex, Chamomile tea, Characterizations, Anti-fungal.*

I. INTRODUCTION

With the significant development of medicine and chemistry after the Second World War, synthetic drugs gradually replaced traditional medicine. However, in the last few years, the industry has been re-evaluating these products, increased by the increased use of the population that seeks options for allopathic medicines aiming at a better quality of life (Almeid*et al*., 2011).

The drug resistance of human pathogens is a significant theme reported in developed and developing countries. On the European continent, the consumption of over one ton of antibiotics has led to a significant increase in resistance of bacterial populations, thus causing a severe public health problem (Duarte *et al*., 2007). The quest for new antimicrobial substances from natural sources, including herbal products, has gained importance in pharmaceutical companies and scientific research (Penna*nt al*., 2001).From time immemorial, there has been growing interest in plant-based traditional medicines, which has made way for extensive research on the potential of natural substances. Many pieces of research have been conducted to analyse and determine the effect of natural chronic diseases.

The chamomile plant is one of the most ancient herbs known to man. It is a member of the AsteraceaeCompositae family and is represented by two standard varieties. The German chamomile and Roman chamomile contain mainly flavonoids, alkaloids, tannins and terpenoids, contributing to their medicinal properties. Many different preparations of chamomile have been developed, the most of which are in the form of herbal tea consumed more than a million cups per day (Janmejai*et al*., 2010). Chamomile has been used as a herbal medication since ancient times. It is still popular today and probably will continue to be used in the future because it contains various bioactive phytochemicals that could provide therapeutic effects. For example, chamomile can help improve cardiovascular conditions and stimulate the immune system.The use of medicinal herbs and herbal medicines is an age-old tradition, and the recent progress in modern therapeutics has stimulated the use of natural products worldwide for diverse ailments and diseases (Miraj, 2016). This study focuses on the formation of a metal complex using used chamomile herbal tea extract in the development of medicine with regards to evaluating its

ISSN No:-2456-2165

curative and preventive properties as it serves as an antifungal agent.

This research work is aimed at extracting, synthesising, and characterising Copper (II) and Iron (III) complexes from used herbal tea (chamomile) and their antifungal activities for possible use as cheap raw materials for drug formulation.

II. MATERIALS AND METHODS

A. *Sample Collection*

Used chamomile tea bags were collected from the researchers' residential homes for the purpose of uniformity and used throughout the research.

Fig. 1: Chamomile Tea

B. *Sample extraction*

The used tea bags were opened, and the chaffs poured into a container and soaked with distilled water; the mixture was kept for two (2) days with agitation at 30 minutes intervals to ensure the total removal of all components. The extract was filtered using Whatman no 1 filter paper. The process was repeated for 4 days to ensure the collection of suitable extracts. It was concentrated using a rotary evaporator, heated at a controlled temperature till dryness. The sample was further ground to fine particles size and carefully transferred into a polyethylene bottles and kept for further analysis.

C. *Synthesis of Copper and Iron Complexes*

Exactly 6g of the powdered extract was dissolved in 40ml of distilled water and filtered. In addition, 3g of the metal salt of Copper chloride and Iron Chloride wereeach dissolved in 40ml of distilled water. The dissolved copper chloride and iron Chloride were added to the filtrate, respectively, with observed colour change. Hydrogen peroxide, 2ml was added to the broth mixture for reduction into iron and copper ions and refluxed at 50^{o}C for 30 minutes with observed colour change and cooled immediately. The mixture was allowed to stay overnight. Crystals were formed and carefully removed and dried in the oven for 2hrs at 40^{o}C.Characterization and other chemical analysis were carried out thereafter.

D. *Characterization of Copper and Iron Complexes*

The Copper(II) and Iron(III) complexes of Chamomile tea extracts were determined by their colour, melting point, percentage yield, solubility, phytochemical screening, UV-Vis spectroscopy, Fourier Transform Infrared spectroscopy (FT-IR), X-ray diffraction(XRD) and X-ray fluorescence(XRF) using the methods of Gongden et al (2020).

E. *Anti-Fungal Assay*

The antifungal activity of the tea extract, copper and iron complexes were determined at different concentrations and evaluated on Eczema infection to ascertain its effectiveness on the selected fungi.

III. RESULTS AND DISCUSSION

The physical characteristics of the synthesized complexes are shown in Table 1.

Sample	Cu/Chamomile complexes	Fe/Chamomile complexes
Colour	Blue	Brown
Melting point	142^{o}c	135^{o}c
% Yield	50	50
pH	7.4	6.6
Solubility in Water	Soluble	Soluble
Solubility in Ethanol	Slightly soluble	Slightly soluble
Solubility in Propanol	Slightly soluble	Slightly soluble
Solubility in methanol	Slightly soluble	Slightly soluble

Table 1: Physicochemical characteristics of copper and iron complexes of chamomile

ISSN No:-2456-2165

Constituents	CHAMOMILE EXTRACT	Cu/Chamomile complex	Fe/Chamomile complex
Alkaloid	++	+++	++
Saponins	+	+	+
Tannins	+++	+++	-
Flavonoids	+++	+++	+++
Carbohydrate	+	+	-
Steriods	-	-	-
Terpenes	-	-	-
Anthraquinones	-	-	-
Cardiac glycosides	-	-	-

Table 2: Phytochemical Screening

+present, ++moderately present, +++ highly present, –absent.

The phytochemical result clearly shows that flavonoids are highly present in both extract and complexes due to their solubility in water. Alkaloids and Tannins follow this.

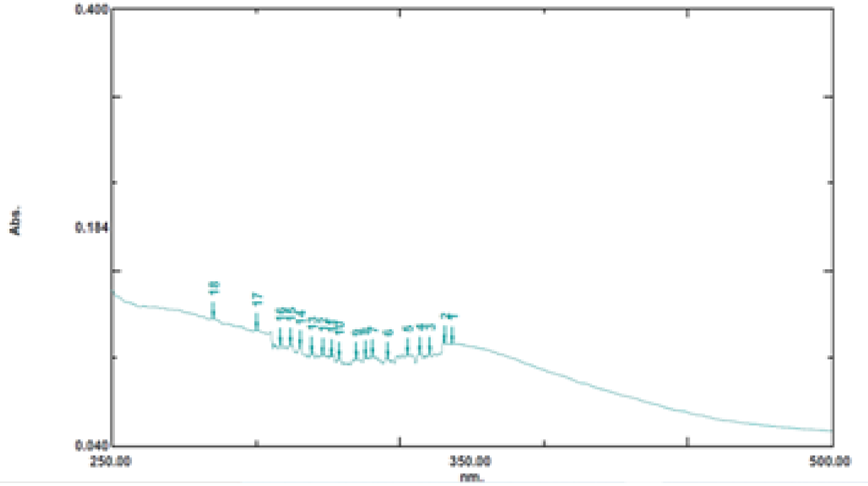

Fig. 2: UV-Vis of Cu (II) Chamomile complex

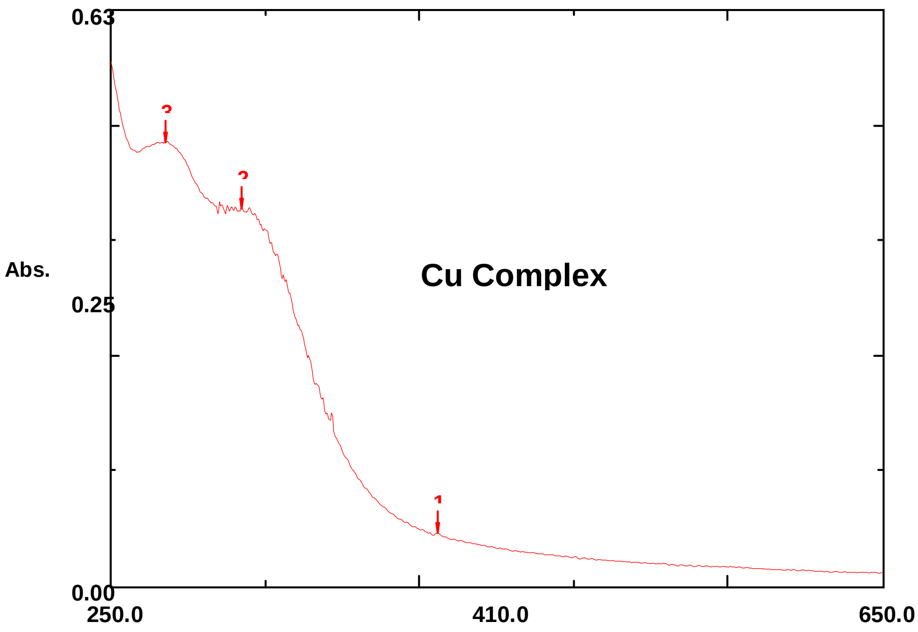

Fig. 3: UV-Vis of Cucl$_2$ salt

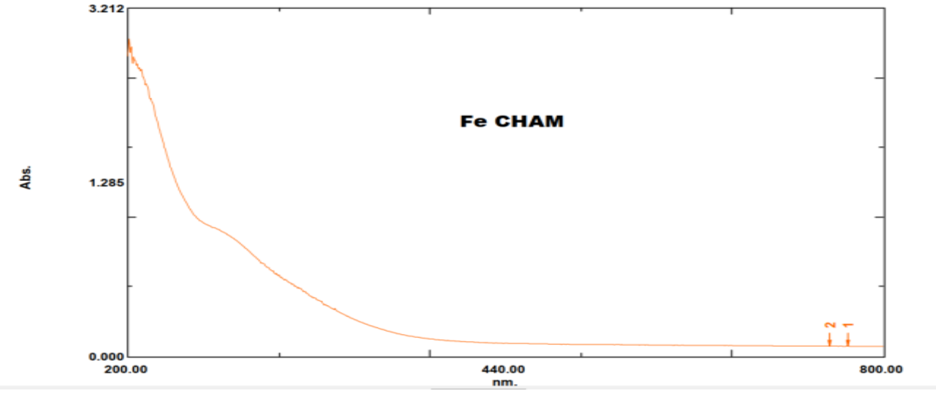

Fig. 4: UV-Vis of Fe (III) Chamomile complex

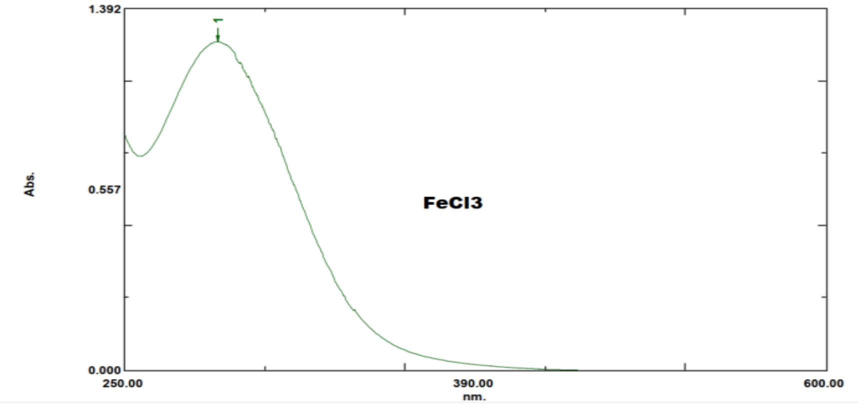

Fig. 5: UV-Vis of Iron (III) chloride

X-ray Fluorescence (XRF) technique wasused to determinethe elemental compositions of the chamomile extract, Cu/chamomile complex and Fe/chamomile complex. Fourteen (14) elements were present with varying percentages from the XRF results.

Analyzing the elements of interest, Fe (extract) has a value of 0.4366%, while Cu/complex with 0.14235 and a significant increase in the Fe/complex with 11.7202%. That is Fe element = Fe/chamomile content > extract content > Cu/chamomile content Cu (extract) has a value of 0.0013% and a great increase of 7.2307% in the Cu/complex with no percentage content in Fe/ chamomile complex that is Cu/complex content > Extract content. This shows a significant change in the elementary level in both extract and complexes.

Elements	**Extract content (%)**	**Cu/complex content (%)**	**Fe/ complex content (%)**
Mg	6.1174	3.3044	5.7610
Si	7.3199	11.7855	14.1674
P	0.4787	0.5793	0.5738
S	0.3556	3.0935	0.0269
K	13.9664	0.8525	3.8988
Ca	2.8621	0.1760	0.2051
Fe	**0.4366**	**0.1423**	**11.7202**
Sr	0.0151	-	-
Nb	0.0239	0.0054	-
Mo	0.0226	-	-
Ag	0.0082	-	-
Cd	00581	0.0044	-
Sb	0.0040	0.0023	-
Cu	**0.0013**	**7.2307**	-

Table 3: XRF Analysis of Extract and Complexes

The FT- IR spectral of both the free ligand and complexes are shown Table 4. The infrared spectrum of the free ligand was compared with that of complexes to determine the coordination sites that may have been involved in the chelation.

Class of compound	Absorption	Assignment
Alcohols, phenols	3400.62	O-H stretch
Alkanes	2926.11	C-H asymmetric stretch
1º amine	1637.62	N-H bend
Aromatics	1400,37	C-C stretch (in ring)
Aromatics amines	1259.56	C-N stretch
Aliphatic amines	1128.39, 1041.60	C-N stretch

Table 4: FTIR Absorption Spectra of Chamomile Extract

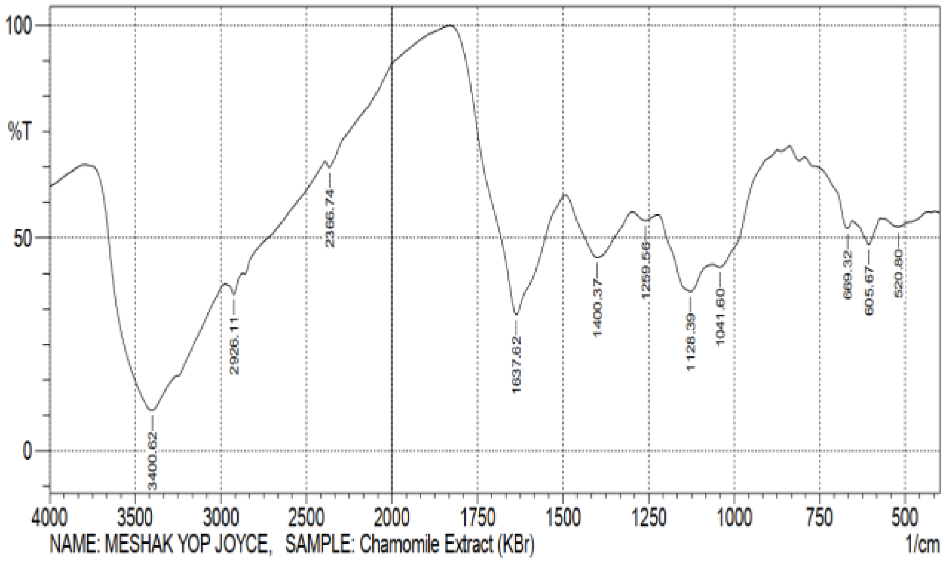

Fig. 6: FTIR of Chamomile Extract

The Fourier Transform Infrared Spectroscopy analysis was performed to identify the chamomile extract's possible biomolecules (functional group). The result observed from chamomile extract gave the IR bands at 3400.62cm^{-1}, 2926.11 cm^{-1}, 1637.62cm^{-1}, 1400.37cm^{-1}, 1259.56 cm^{-1}, 1128.39 cm^{-1} and 1041.60 cm^{-1}. The absorption at 3400.62cm^{-1} is due to the stretching of the hydroxyl group (O-H) present in the extract, the band positioned at 2926.11cm^{-1} may be due to C-H's symmetric stretching of saturated (sp3) carbon. The band at 1637.62cm^{-1} could be attributed to the N-H bending mode of absorbed water; the band at 1400.37cm^{-1} is due to C-C stretch (in ring). A notable band at 1259.56 cm^{-1} can be assigned to C-N in aromatic amines. The band that appeared at 1128.39 cm^{-1} and 1041.60 is due to the C-N stretch in aliphatic amines.

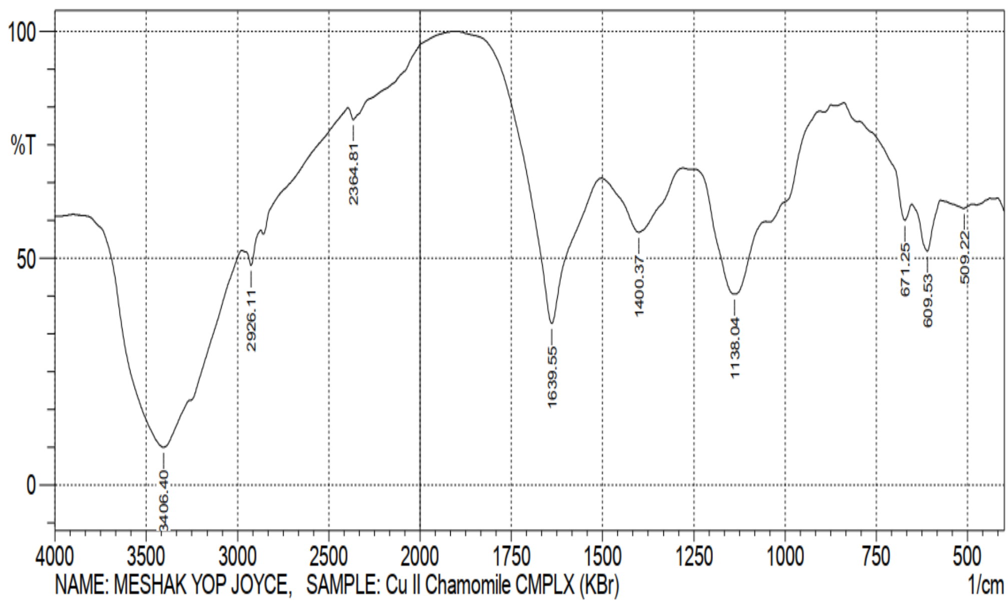

Fig. 7: FTIR of Cu (ii) Chamomile Complex

ISSN No:-2456-2165

The Fourier Transform Infrared Spectroscopy analysis was performed to identify the possible biomolecules (functional group) present in the Cu (II) chamomile complex (KBr). The result observed gave the IR bands are 3406.40 cm^{-1}, 2926.11 cm^{-1}, 1639.55 cm^{-1}, 1400.37cm-1, 1138.04 cm^{-1}. The absorption at 3406.40 cm^{-1} is due stretching of hydroxyl (O-H) groups that are present in the complex, the band positioned at 2926.11 cm^{-1} correspond to the asymmetric C-H stretch of hydrocarbons, a sharp intensity band observed at 1639.55 cm^{-1} is assigned to N-H vibration, the band which appeared at 1400.37 cm^{-1} could be attributed to C-C stretch (in-ring), while the band that appeared at 1138.04 cm^{-1} is due to C-N stretching of aliphatic amines.

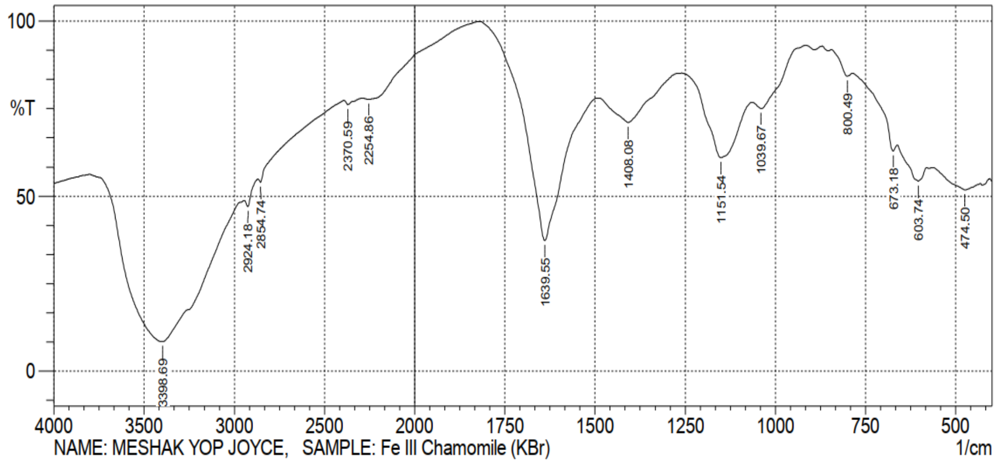

Fig. 8: FTIR of Fe (III) chamomile complex

The Fourier Transform Infrared Spectroscopy analysis was performed to identify the functional groups in Fe (III) chamomile (KBr). The result observed gave the IR bands at 3398.69 cm^{-1}, 2924.18 cm^{-1}, 285474 cm^{-1}, 1639.55 cm^{-1}, 1408.08 cm^{-1}, 1151.54 cm^{-1}, 1039.67 cm^{-1}, 800.49 cm^{-1},673.18 cm^{-1}, 603.74 cm^{-1} and 474,50 cm^{-1}. The band at 3398.69 was due to the O-H stretch present in the complex, while the absorption at 2924.18 cm^{-1} and 2854.74 cm^{-1} correspond to the asymmetric and symmetric C-H stretches (CH_3 and CH_2) of the hydrocarbons. The band at 1639.55 cm^{-1} could be an N-H bend of 1° amines, and the band positioned at 1408.08cm^{-1} may be due to C-C stretch (in ring). The band at 1151.54 cm^{-1} is due to the C-H wag, while the band observed at 1039.67 cm^{-1} is due to the C-N stretch in aliphatic amines.

XRD analysis is a powerful technique that studies a sample's crystalline nature. X-ray diffraction characterized these samples at room temperature to describe a material's atomic arrangement and determine the position and intensity of diffraction peaks in an X-ray scattering pattern. The diffraction pattern of complexes was recorded between 2θ, ranging from 0° to 70°. The crystalline size of the samples is estimated using the Scherer's formula $D=K\lambda / \beta 2\theta \cos\theta$, here K is constantly taken to be 0.94, λ the wavelength of X-ray used (λ=0.15406nm), and β2θ full width of half maxima of all peaks of the XRD pattern and θ is Bragg angle. The diffraction patterns have been successfully indexed. The crystalline size was found for copper and iron chamomile complexes as 73.1nm and 37.15nm, respectively. It was observed that the copper/chamomile complex is more crystalline than the iron/chamomile complex.

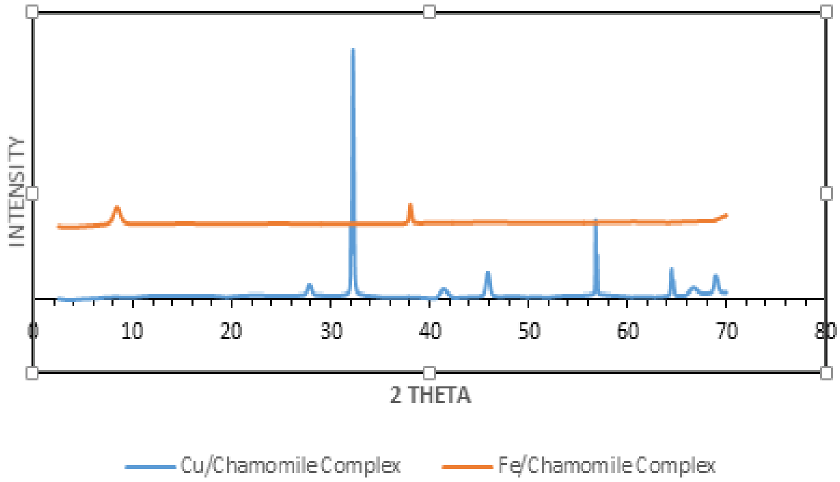

Fig. 9: XRD for combined complexes.

IV. ANTIFUNGAL ACTIVITY OF LIGAND (CHAMOMILE) AND COMPLEXES

Antifungal activity of ligand (chamomile) and metal complexes were tested in vitro against fungal such as *Aspergillusflavus, Aspergillusniger, Fusariumspp* and *Penicilliumspp* by paper disc plate method. The compounds were tested at concentrations (mm/mg) of 12.5, 25, 50, 100, and 200 in DMSO and compared with known antibiotics, Nystatin (see Table 6). From the table below, it is clear that the inhibition by the metal complex is higher than that of the ligand (chamomile), and the microorganism was destroyed at a higher concentration. The results agree with previous findings concerning the comparative activity of the free ligand and its complexes. Such enhanced activity of the metal complex is due to the increased hydrophilic nature of metal ions in complexes. The increase in activity with concentration is due to the effect of metal ions on the average cell process.

Microorganism used	**Test compounds**														
	Li(chamomile) Concentration mm/mg					Li-Cu complex Concentration mm/mg					Li-Fe complex Concentration mm/mg				
	200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
Aspergillusniger	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+
Aspergillusflavus	-	+	+	+	+	-	-	-	+	+	-	-	+	+	+
Fusariumspp	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+
Penicilliumspp	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+

Table 6: Antifungal Activity of Ligand (Chamomile) and Complexes

Key: Li= Ligand -= No growth += There is growth on the plate

The result shows that copper is a better antifungal agent than Iron and that the complexes are biologically active, and show enhanced antimicrobial activities compared to the ligand.

V. CONCLUSION

A simple, economic and environmentally benign chamomile tea for synthetic method has been developed for preparing copper (II) and iron (III) complexes. The phytochemical analysis was assessed, and the result dictated the presence of flavonoids, alkaloids and tannins with the absence of tannins in the iron complex, which is an indication that Iron is used to dictate the presence of tannins. The products were characterized by UV-Vis, XRD, FT-IR and XRF analysis. The UV-Vis analysis confirms chromophores' presence, indicating the complexes were prepared successfully. The XRD pattern showed that Cu and Fe synthesized complexes were calculated using thee Scherer's equation; these calculations are used to observe the crystalline size of the complexes, and copper shows more crystallinity than Iron with 73.2nm and 37.2nm, respectively. XRD spectrum confirmed that the synthesized complexes were crystalline, and the high peaks in XRD indicate the active composition of the synthesized complexes. The IR stretching frequency of the ligand's Aromatic amines (C-N) in the range 1259.56cm-1 disappeared in the complexes.

REFERENCES

[1.] Almeida MR, Pinto AC. (2011). Uma brevehistória da química Brasileira. Cienc

[2.] Andrade S.F. (2017). Antifungals discovery: an insight into new strategies to combat antifungal resistance.

[3.] Duarte MCT, Leme EE, Delarmelina C, Soares AA, Figueira GM, Sartoratto A. (2007). The activity of Essential Oil from Brazilian Medicinal Plants on Escherichia coli. J Ethno Pharmacol.; 11(2):197-201.

[4.] Janmejai K. Srivastavaet al., (2010): chamomile: A herbal medicine of the past with bright future.

[5.] Miraj S, Samira Alesaeidi; (2016): A systematic review study of therapeutic effect of MatricariaRecutita chamomile (chamomile). 8(9); 3024-3031.

[6.] Penna C, Marino S, Vivot E, Cruañes MC, Muñoz JD, Cruañes J, Ferraro G Gutkind G, Martino V; (2001) Antimicrobial activity of Argentine plants used in the treatment of infectious diseases. Isolation of active compounds from Sebastianiabrasiliensis. J Ethnopharmacol.; 77:37-40

[7.] RavikumarChandini (2018): Review on Herbal teas. BDS Student, savitha Dental College, Chennai J. pharm. Sci. &Res. 6(5): 236-238.

[8.] Nnamani, J.O, Nasiru, P.Y, Wilson, L.D, Mohammed, U. (2020): Synthesis, Characterization and Evaluation of antimicrobial potency of Fe(II), Mn(II), Cu(II), Co(II) and Zn(II) ion complexes with Schiff base, Derived from salicyladehyde and p.toluidine.World Journal of innovative research 8(2): 16-21.