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Identification of Bioactive Compounds in Methanolic Extracts of Some Selected Plants by Infrared Spectroscopy

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ABSTRACT

Background and Objective: When two or more herbs are used in formulations, they are known as polyherbal formulations. This study evaluated the bioactive ingredients in some selected herbs commonly used in polyherbal mixture formulations by infrared spectroscopy. **Materials and Methods:** Fresh samples of *Vernonia amygdalina* leaves, *Allium sativum, Garcinia kola, Curcuma longa and Zingiber officinale* were purchased from Wukari New Market, Taraba State, Nigeria. The methanolic extract of the samples was obtained and Fourier Transform Infrared Spectrophotometer (FTIR) analysis was conducted on the extracts. **Results:** The FTIR spectra of the extracts showed bands and wave numbers of between 3280 cm^{-1} and 991 cm^{-1} as the prominent peaks. The peaks at these frequencies were strong, broad and medium, which confirmed the presence of compounds with the functional groups of alkanes, alkenes, amines, carboxylic acids and alcohols in the extracts. **Conclusion:** All the samples showed a consistent presence of similar classes of organic compounds. The results of the study confirmed that the selected herbs are vital wellsprings of phytochemicals that offer conventional therapeutic treatment for different infirmities.

KEYWORDS

FTIR, phytochemicals, *Allium sativum*, *Garcinia kola*, *Curcuma longa*, *Zingiber officinale*

INTRODUCTION

For thousands of years, Chinese physicians have utilised herb-herb combinations, often known as polyherbal treatment, although there is little scientific proof of their medicinal advantages[1]. In comparison to a single medicine, a pharmacological combination frequently shows promise in the treatment of disorders. In Western medicine, the idea of pharmacological combinations is well-established and it has seen a lot of success over the years.

Drug combination therapies for infectious illnesses and cancer have given patients fresh hope in recent years[2]. It has been demonstrated that both naturally occurring herbs and herbal components combined into specific formulae may interact. These include mutually beneficial interactions, mutual support, mutual restriction and antagonistic interactions[3]. The majority of polyherbal substances are utilised in the

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Ayurvedic medical system to treat a variety of illnesses. *Plumbago zeylanica*, *Picrorrhiza kurroa*, *Piper nigrum*, *Zingiber officinale*, *Sodii carbonas impura*, *Phyllanthus emblica*, *Terminalia chebula*, *Calcii oxidum Potassii* and *carbonas impura* make up Hepax-A polyherbal[4]. Ayurvedic doctors frequently recommend Indukantha Ghritha (IG), a polyherbal remedy made up of 17 plant components, for a variety of diseases[5].

As a result of the incredible clinical success of its formulations, the Unani system of medicine is also gaining popularity on a global scale. Although unani remedies have been used for a long time, there is no evidence to support either their usefulness or safety. The absence of review has therefore hindered the creation of rules and laws[6]. The Majoon Suranjan (MS) is a polyherbal formulation used in the Unani system of medicine for the treatment of rheumatoid arthritis that contains *Lawsonia inermis*, *Foeniculum vulgare*, *Capparis spinosa*, *Terminalia chebula*, *Ipomoea turpethum*, *Zingiber officinalis*, *Convulvulus scammony*, *Colchicum luteum*, *Cassia angustifolia*, *Piper nigrum*, *Coriandrum sativum*, *Rosa damascus*, *Origanum vulgare*, *Pyrethrum indicum*, *Plumbago zelanicum*, *Verbascum thapus* and *Ricinus communis*. A successful attempt has been made to create a polyherbal formulation combining the leaf extracts of *Glycosmis pentaphylla*, *Tridax procumbens* and *Mangifera indica*, which was then tested for antiarthritic activity[7].

Studies revealed that some plants (*Vitis vinifera*, *Phyllanthus emblica* L., *Punica granatum*, *Cinnamomum cassia*, *Ginkgo biloba* L. and *Camellia sinensis* Linn.) had high concentrations of phenolics and flavonoids and that the polyherbal combination of these plants and green tea had the highest level of antioxidant activity than all the individual extract[8]. Because of their synergism and less adverse effects, herbal mixtures (polyherbal) are often preferred over single herbs in most traditional methods for managing diabetes[9]. The polyherbal formulation used to prepare the diabetic wound area was shown to be equally as safe and effective in treating diabetic foot ulcers as the common silver sulphadiazine cream[10].

The anti-inflammatory activity of the polyherbal formulation Entox® consist of *Allium cepa*, *Allium sativum*, *Aloe vera*, *Cajanus cajan*, *Coccinia indica*, *Caesalpinia bonducella*, *Ficus bengalenesis*, *Gymnema sylvestre*, *Momordica charantia*, *Ocimum sanctum*, *Pterocarpus marsupium*, *Swertia chirayita*, *Syzigium cumini*, *Tinospora cordifolia* and *Trigonella foenum-graecum* was investigated in rats for acute and sub-acute models of inflammation using carrageenan-induced rat paw edema and cotton pellet granuloma methods respectively at a dose of 300 mg kg^{-1} and 600 mg kg^{-1} administered orally. In all animal models, the formulation significantly reduced inflammation and its anti-inflammatory effects were equivalent to those of the widely used medication indomethacin[11]. The BHUx, a proprietary polyherbal formulation made from the aqueous fraction of five ayurvedic medicinal herbs, significantly reduces inflammation by inhibiting cyclooxygenase-2 and lipoxygenase-15[12]. The polyherbal formulation "Bhāra gyādi" containing ingredients such as *Clerodendrum serratum*, *Hedychium spicatum* and *Inula racemosa,* was known to possess antioxidant activity[13].

In an attempt to increase the horizon of polyherbal formulations, *Vernonia amygdalina* leaves, *Allium sativum*, *Garcinia kola*, *Curcuma longa* and *Zingiber officinale* were investigated for their bioactive components by FTIR spectroscopy in this study. This investigation was aimed at facilitating the formulation of a polyherbal mixture from these plants for efficacy and mechanistic analysis through animal studies.

MATERIALS AND METHODS

Study area: The study was carried out in the Department of Biochemistry, Federal University Wukari, Taraba State Nigeria from April, 2021 to April, 2022.

Collection of plant material: Fresh samples of the *Vernonia amygdalina* leaves, *Allium sativum*, *Garcinia kola*, *Curcuma longa* and *Zingiber officinale* were purchased from the Wukari New market, Taraba State, Nigeria.

Preparation and extraction of plant material: The samples were air-dried for five days. The dried samples were coarsely pounded using a pestle and mortar and stored in tightly covered glass jars for methanolic extraction. The powdered material of the plant parts was soaked in 500 mL of methanol for 24 hrs at room temperature. The extracts were filtered through Whatman No. 1 filter paper. After the filtration, the filtrates were concentrated to dryness and kept in a refrigerator for the spectroscopy analysis.

Fourier Transform Infrared Spectrophotometer (FTIR): In order to prepare a translucent sample disc, exactly 10 mg of the extract was encapsulated in 100 mg of KBr pellet. The encapsulated sample disc was loaded in the FTIR spectroscope of a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} according to Pakkirisamy *et al.*[14].

RESULTS

In the present study, the biochemical contents of five different plants were investigated using FTIR spectroscopy by monitoring different functional groups. The spectra were shown in the figures while the interpretations were given in the tables.

FTIR spectroscopy of *Garcinia kola*: The FTIR spectrum of *Garcinia kola* in Fig. 1 has bands and wave numbers of 3280 cm^{-1} to 2926 cm^{-1} as the prominent peaks. The peaks at the frequencies of 3280 cm^{-1} to 995 cm^{-1} were strong, broad and medium. The present FTIR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extract of *Garcinia kola* in Table 1.

FTIR spectrum of *Vernonia amygdalina*: In the FTIR spectrum showed in Fig. 2, the leaf extract of *V. amygdalina* has bands and wave numbers of 3280 cm^{-1} to 2922 cm^{-1} as the prominent peaks. The peaks at the frequencies of 3280 cm^{-1} to 1017 cm^{-1} were strong, broad and medium. The results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extracts of *V. amygdalina* as shown in Table 2.

FTIR spectrum of *Allium sativum*: Considering the FTIR spectrum showed in Fig. 3, the extract of *Allium sativum* has bands and wave numbers of 3276 cm^{-1} to 2855 cm^{-1} as the prominent peaks. The peaks at the frequencies of 3276 cm^{-1} to 1006 cm^{-1} were strong, broad and medium. Table 3 confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extract of *Allium sativum*.

FTIR spectrum of *Zingiber offinale*: *Zingiber offinale* extract has bands and wave numbers of between 3280 cm^{-1} to 2922 cm^{-1} as the prominent peaks while those peaks between the frequencies of 3280 cm^{-1} to 991 cm^{-1} were strong, broad and medium as demonstrated in Fig. 4. The FTIR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extracts of *Zingiber offinale* in Table 4.

FTIR spectrum of *Curcuma longa*: Figure 5 showed that *Curcuma longa* has bands and wave numbers of between 3628 cm^{-1} to 2922 cm^{-1} as the prominent peaks. The peaks between the frequencies of 3628 cm^{-1} to 998 cm^{-1} were strong, broad and medium. The presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extract of *Curcuma longa* was obvious in Table 5.

FTIR spectrum of the plant mixture: The FTIR spectrum in Fig. 6 showed that the extract of the combined mixture has bands and wave numbers of between 3291 cm^{-1} to 2922 cm^{-1} as the prominent peaks. The peaks between the frequencies of 3291 cm^{-1} to 1002 cm^{-1} were strong, broad and medium. The present FTIR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extract of the combined mixture in Table 6.

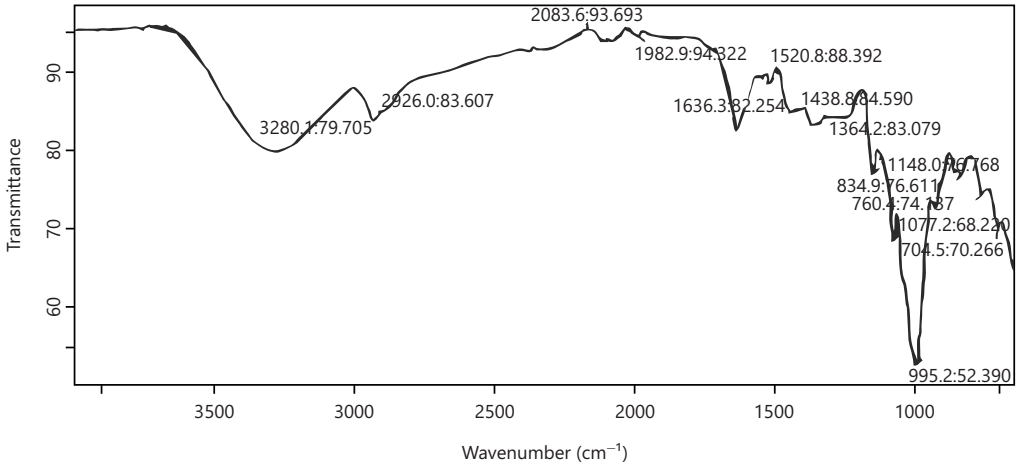

Fig. 1: FTIR spectrum of methanolic extract of *Garcinia kola*

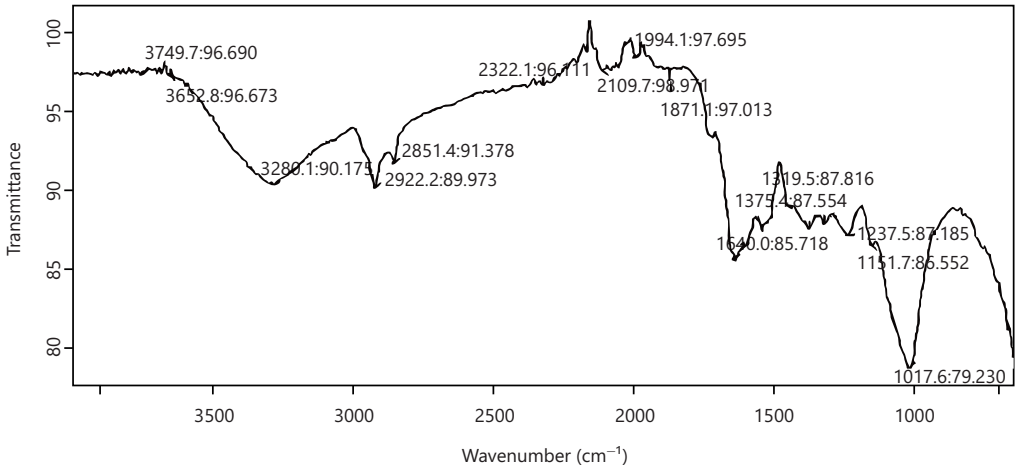

Fig. 2: FTIR spectrum of methanolic extract of *Vernonia amegdalina*

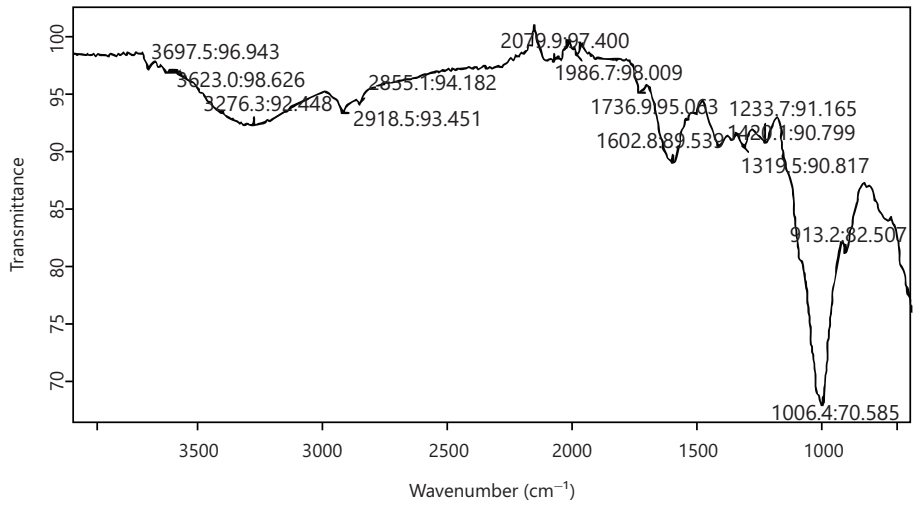

Fig. 3: FTIR spectrum of methanolic extract of *Allium sativum*

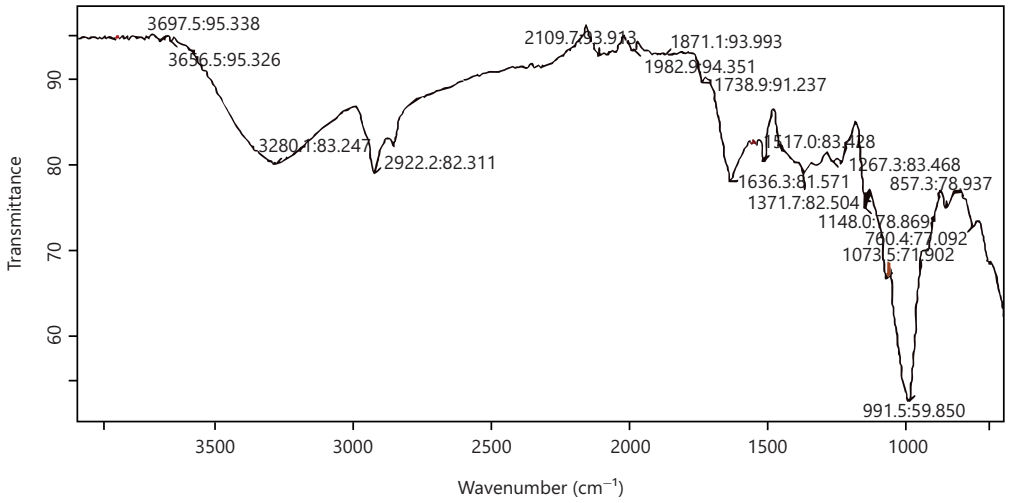

Fig. 4: FTIR spectrum of methanolic extract of *Zingiber officinale*

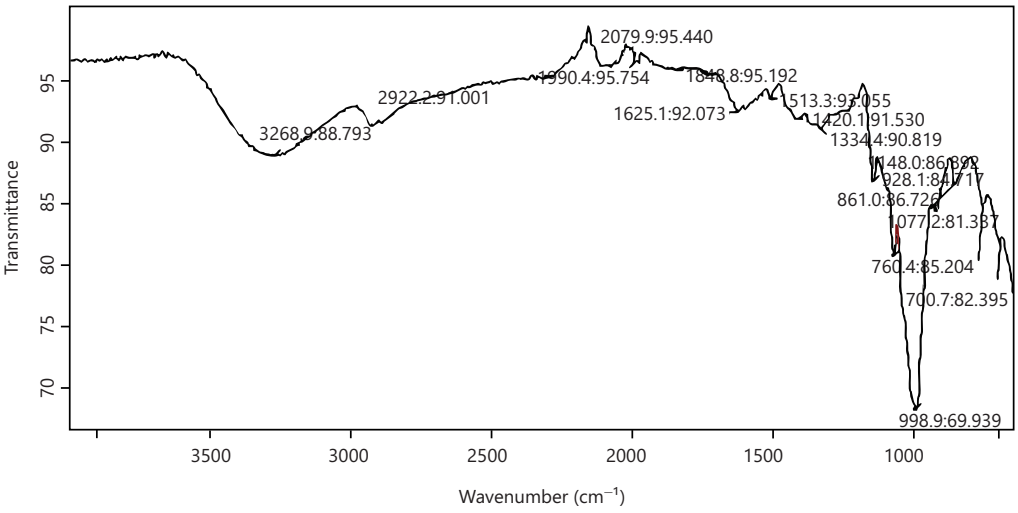

Fig. 5: FTIR spectrum of methanolic extract of *Curcuma longa*

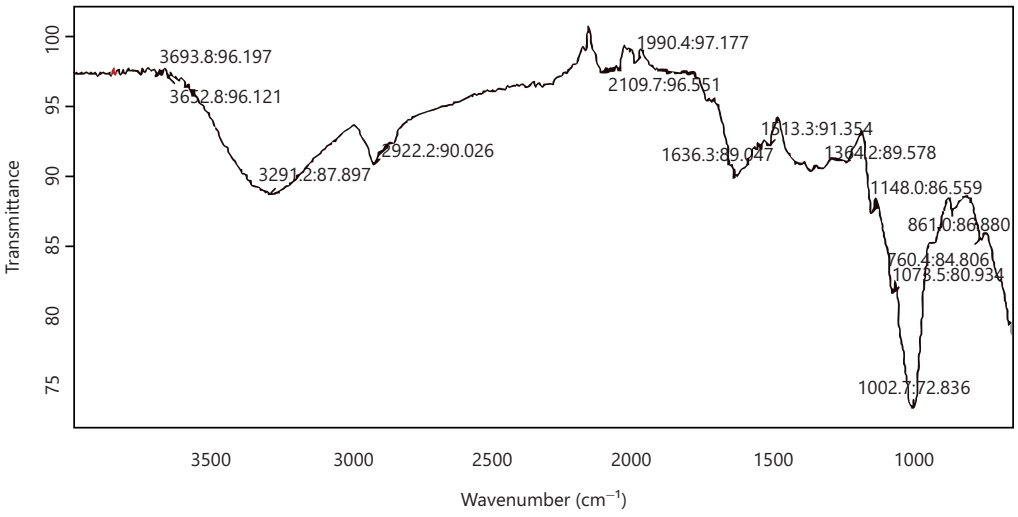

Fig. 6: FTIR spectrum of methanolic extract of the mixture of the five plants

Table 1: Bioactive components from *Garcinia kola* FTIR spectrum

Wavelength number (cm^{-1})	Functional group	Inference
3280	O-H stretch, H-bonded	Alcohols, phenols
2986	C-H stretch	Alkanes and alkyls
1982	C=O stretch	Carbonyls (general)
1636	C=O stretch	Carboxylic acids
1520	C-C stretch (in-ring)	Aromatics
1148	C-N stretch	Aliphatic amines
1077	C-N stretch	Aliphatic amines
995	=C-H bend	Alkenes

Table 2: Bioactive components from *Vernonia amegdalina* FTIR spectrum

Wavelength number (cm^{-1})	Functional group	Inference
3749	O-H stretch, free hydroxyl	Alcohols, phenols
3280	O-H stretch, H-bonded	Alcohols, phenols
2922	C-H stretch	Alkanes
2322	C≡N stretch	Nitriles
2109	C≡C stretch	Alkynes
1640	-C=C- stretch	Alkenes
1375	CH_3C-H bend	Alkanes and alkyls
1017	C-F stretch	Alkyl halides

Table 3: Bioactive components from *Allium sativum* FTIR spectrum

Wavelength number (cm^{-1})	Functional group	Inference
3697	O-H stretch, free hydroxyl	Alcohols, phenols
3276	N-H stretch 1°, 2°	Amines, amides
2918	O-H stretch	Carboxylic acids
1986	C-O stretch	Alcohols
1602	C-C stretch (in-ring)	Aromatics
1319	C-O stretch	Alcohols, carboxylic acids, esters, ethers
1006	C-F stretch	Alkyl halides
913	O-H bend	Carboxylic acids

Table 4: Bioactive components from *Zingiber officinale* FTIR spectrum groups

Wavelength number (cm^{-1})	Functional group	Inference
3697	O-H stretch, free hydroxyl	Alcohols, phenols
3280	O-H stretch, H-bonded	Alcohols, phenols
2922	C-H stretch	Alkanes
2109	-C≡C- stretch	Alkynes
1982	C-O stretch	Alcohols
1636	N-H bend	1° amines
1517	N-O asymmetric stretch	Nitro compounds
1371	C-H rock	Alkanes
1148	C-O stretch	Alcohols
1073	C-O stretch	Alcohols
991	=C-H bend	Alkenes

Table 5: Bioactive components from *Curcuma longa* FTIR spectrum

Wavelength number (cm^{-1})	Functional group	Inference
3268	N-H stretch	1°, 2° amines, amides
2922	O-H stretch	Carboxylic acids
1990	C=O stretch	Carbonyls (general)
1632	N-H bend	1° amines
1513	N-O asymmetric stretch	Nitro compounds
1334	C-N stretch	Aromatic amines
1148	C-O-C stretch	Ethers
1077	C-O stretch	Alcohols
998	=C-H bend	Alkenes
700	-C≡C-H: C-H bend	Alkynes

Table 6: Bioactive components from the FTIR spectrum of the mixture of the five plants

Wavelength number (cm^{-1})	Functional group	Inference
3693	O-H stretch, free hydroxyl	Alcohols, phenols
3291	N-H stretch	1°, 2° amines, amides
2922	C-H stretch	Alkanes
2109	-C≡C- stretch	Alkynes
1990	C-O stretch	Alcohols
1636	N-H bend	1° amines
1513	N-O asymmetric stretch	Nitro compounds
1364	C-H rock	Alkanes
1148	C-O-C stretch	Ethers
1002	C-N stretch	Aliphatic amines
760	C-Cl stretch	Alkyl halides

DISCUSSION

Each functional group present in the methanol extract of the selected plant samples produced a characteristic absorption pattern at the functional group regions associated with wavenumber ranges from 3200-3600 cm^{-1} for O-H stretching in alcohols and carboxylic acids, 2900-3000 cm^{-1} for C-H stretching in alkanes and alkyl groups, 1700-1750 cm^{-1} for C=O stretching in carbonyl compounds such as aldehydes, ketones and carboxylic acids, 1600-1650 cm^{-1} for C=C stretching in conjugated alkenes and aromatic compounds,1400-1450 cm^{-1} for C-H bending in alkanes and alkyl groups, 1200-1300 cm^{-1} for C-N stretching in amines and amides and 1000-1100 cm^{-1} for C-O stretching in ethers, esters and carboxylic acids. The peak shapes exhibited by the functional groups at the functional group regions were usually broad whereas that at the fingerprint regions were narrow. On the other hand, the peak intensities were mild/weak at the functional groups regions while they showed more strong intensities especially at 1000 cm^{-1} wavenumber of the fingerprint regions.

Herbs are an essential source of phytochemicals that provide traditional medical therapy for a variety of ailments[15]. The primary source of functional components for the development of cutting-edge chemotherapeutic medicines is plants[16]. Infrared radiation is used in Fourier Transform Infrared (FTIR) spectroscopy, a vibrational spectroscopic technique, to cause molecular bonds in the material that absorbs it to vibrate. The majority of the samples have various subatomic bonds or unique arrangements of subatomic bonds. Data on the substances and particles inside the specimen were provided by the FTIR[17]. Based on the peak values in the area of IR radiation, the FTIR spectrum was used to identify the functional groups of the active components contained in the extracts. When the concentrate was introduced to the FTIR, the functional groups of the components were separated, providing a clear glimpse of the pinnacle proportion of the bioactive ingredients in the extracts.

The findings of this study are consistent with those of earlier investigations on different plants[18-21], in which it was discovered that substances such as nitro compounds, 1°, 2° amines, amides, esters and aliphatic amines were present. Similar to this, Lanjwani *et al.*[22] earlier screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons and halogens that are responsible for the various medicinal characteristics of *Ricinus communis*. Equally, in an ethylacetate leaf extract of *Abutilon indicum*, Saranya and Sekar[23] employed FTIR to pinpoint functional group components of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids and aromatics. Additionally, FTIR analysis of methanol leaf extracts of *Senna auriculata*, *Phyllanthus maderaspatensis*, *Solanum torvum* and *Phyllanthus amarus* confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones and alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds, which showed major peaks[24].

The plants gained therapeutic properties due to the presence of phenolic chemicals in them[25]. The fact that phenols and flavonoids have Hydroxyl groups (OH^{-}) that are chemically linked directly to the benzene ring makes them the main antioxidants. This makes it simple for them to provide electrons to free radicals

that are in need of them, lessening the threat they pose to the biological system[26]. Additionally, esters are a key factor in the synthesis of fragrance in plants since they are generally created by esterifying alcohol with fatty acids[27]. Amines have also been shown to be flavour precursors, therefore certain plants have the flavour that is linked with them[28]. The OH group was discovered to be present consistently in the methanol extracts of all the plants among the functional groups revealed in the extracts. The presence of the OH group, which has a propensity to generate hydrogen bonds, may imply that the methanol extract has a greater potential for inhibiting the growth of microbes[29].

This study implied that the methanol extract of *Vernonia amygdalina* leaves, *Allium sativum*, *Garcinia kola*, *Curcuma longa* and *Zingiber officinale* investigated for their bioactive components showed the presence of promising functional groups of antioxidant molecules and hence the selected plants could be utilized for the formulation of viable agents against oxidative stress. On the contrary, the results of this research may have been affected by interferences of other substances in the samples or in the environment that absorb infrared radiation at the same wavenumber as the samples. Therefore, the selected plants should be purified for specific bioactive components to be fully utilized in herbal medicine.

CONCLUSION

The findings of the current study demonstrated the value of FTIR spectroscopy as a method for characterizing and analyzing the various biomolecules found in plant extracts. The study also comes to the conclusion that the five different plants included in the FTIR analysis show distinct differences among their biomolecules. The comparative analysis of the plants showed significant variance and it may be used to identify the plant that contained the highest concentration of phytoconstituents, which are plant-based treatments for a variety of ailments. The study's findings show that these plants are crucial for medicine and that they may be further investigated for their efficacy and pharmacological effects in order to harness their potentials the pharmaceutical sector.

SIGNIFICANCE STATEMENT

This study discovered the presence of compounds with the functional groups of alkanes, alkenes, amines, carboxylic acids and alcohols in the extracts and their mixture that can be beneficial for polyherbal formulations for the treatment and management of different diseases. This study will help the researchers to uncover the critical areas of pharmacologic effect of their polyherbal mixture that many researchers were not able to explore. Thus a new theory on the mechanism of action may be arrived at.

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