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Unveiling the phytochemical composition, antioxidant potencies and bioactive compounds of *biebersteinia odora* Stephan, an underexplored plant of the Trans Himalayas

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ABSTRACT

Biebersteinia odora (family Biebersteiniaceae), an aromatic perennial herbaceous plant, is traditionally, a significant medicinal plant, valued by various cultures for its medicinal properties. The present study explores the phytochemical composition and antioxidant potential of the aqueous extract of B. odora. The analysis revealed that at its 1% concentration, the total phenolic, flavonoid, and tannin content were 5.97 mg FAE g^{-1}, 0.34 mg QE g^{-1}, and 121.53 mg TAE g^{-1}, respectively. The total antioxidant capacity ranged between 0.05 and 0.53 mg AAE g^{-1}. In vitro antioxidant assays revealed concentration-dependent scavenging potential of the extract. Further, GCMS identified 21 putative compounds with strong pharmacological potential and FTIR spectra revealed the presence of functional groups with OH as the major functional group. Based on the study, it is established that B. odora possesses a strong phytochemical composition and significant antioxidant capabilities, providing valuable insights into its therapeutic potential and supporting its role in holistic healing practices.

Keywords: antioxidants, ethnomedicine, flavonoids, phenolics, phytochemicals, secondary metabolites, tannins

INTRODUCTION

In the healthcare domain, the often underestimated valuable source of wisdom lies within indigenous cultures, where ethnomedicinal knowledge rooted from natural resources, plays a vital role in traditional healing practices (Alves and Rosa, 2005). It works as a bridge between the past and the future and offers a deep understanding of traditional healing methods while holding the promise of enhancing and advancing healthcare in today's world and beyond (Mahapatra *et al.*, 2019). However, despite the value of this indigenous medicinal knowledge, it faces several challenges, such as inadequate documentation, limited research, and the complexities of integrating traditional practices with modern medical approaches (Eyong, 2007). As a result, this valuable knowledge is slowly disappearing. Given the importance, there has been a growing effort to document the knowledge to align it with current scientific theories and practices (Kala *et al.*, 2006). This will not only protect the cultural heritage of indigenous communities but also provide an opportunity to access a vast source of information that can improve modern healthcare practices (Kala *et al.*, 2006).

Biebersteinia, a genus belonging to the family Biebersteiniaceae, comprises four distinct species: *B. heterostemon* Maxim., *B. multifida* DC., *B. orphanidis* Boiss, and *B. odora* Stephan ex Fisch (Zhang *et al.*, 2020). These species have a wide distribution range in the mountainous and semi-arid regions, spanning from central Asia to the Mediterranean (Muellner *et al.*, 2007). The *Biebersteinia* species have a rich history of traditional medicinal value, having been employed to address a variety of health conditions. Modern pharmacological research has substantiated the medicinal properties of these plant species, including antioxidant, analgesic, anti-inflammatory, antispasmodic, and hypoglycemic effects (Zhang *et al.*, 2020). Natural products isolated from these plants encompass a diverse range of chemical classes, constituting flavonoids, alkaloids, phenols, terpenoids, sterols, fatty acids, and various constituents found in essential oils (Zhang *et al.*, 2020). Consequently, researchers worldwide have devoted their efforts to uncover the bioactive compounds in *Biebersteinia* species.

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B. odora, in particular, is a perennial aromatic herb with a broad presence across central Asia (Doležal *et al.*, 2018). It is found in the alpine meadows, rocky terrain, and screed slopes at a height of ~ 4800 m asl (Chaurasia *et al.*, 2008). *B. odora* has ferns-like leaves with vibrant yellow coloured flowers (Doležal *et al.*, 2018). The plant has high ethnomedicinal importance as it is used by tribal people in treating various diseases such as migraine and fever, cuts and wounds, skin sore, urinogenital disorders, ophthalmic diseases, peptic ulcer, and diarrhea and also used as a blood purifier and antiseptic (Navchoo and Buth, 1989; Abbas *et al.*, 2017; Angmo *et al.*, 2012). Unlike the rest of the *Biebersteinia* species, *B.odora* has not been analysed for its phytochemical constituents in detail. As *B. odora* holds high pharmacological value as traditional medicine and a significant ethnobotanical importance, there is a need for investigations pertaining to its phytochemical constituents and the possible biological activities. Plants are rich sources of naturally occurring bioactive compounds, often referred to as phytochemicals or secondary metabolites, which serve multiple purposes within the plant, from defending against herbivores to contributing to its pharmacological properties (Tran *et al.*, 2020). These phytochemicals have garnered a significant attention from researchers who aim to extract and utilize them in the development of essential drugs in the allopathic medicine system (Tomasini and Theilade, 2019). In light of the potential offered by *B. odora*, an underutilized but traditionally significant medicinal plant, our current study was designed with primary objectives i.e. to evaluate the phytochemical constituents present in *B. odora* and to assess the *in vitro* antioxidant properties of this plant. This research could potentially unlock valuable insights into harnessing the therapeutic potential of this plant and contribute to the development of novel medicines and treatments.

MATERIALS AND METHODS

The aerial parts of *B. odora* were harvested in July 2021 from the mountainous terrains of Kukshow village in Sham Valley, Ladakh, at ~3487m asl (34°48'84" N and 76°62'42" E). The plant was identified by referencing records from the Botanical Survey of India in Dehradun, and herbarium sheets were submitted with the accession number BSD 426. After collection, the plant material was shade-dried and finely ground into a powder, which was then used to make an aqueous stock solution with a concentration of 1% (*w/v*) using distilled water. Various concentrations, specifically 0.0625, 0.125, 0.25, and 0.5% were subsequently derived from the stock solution for conducting phytochemical analysis of bioactive compounds and *in vitro* antioxidant assays.

The aqueous extract of *B. odora* was subjected to both qualitative and quantitative analyses to identify phytochemicals. To determine the presence or absence of various compounds, standard methods (Kanthal *et al.*, 2014) were used for qualitative analysis. Quantitative analysis was employed to assess the total phenolic (TPC), flavonoid (TFC) and tannin content (TTC) following protocols of Swain and Hillis (1959), Meda *et al.* (2005), and Makkar *et al.* (1993) respectively, and the results were expressed as ferulic acid equivalent (mg FAE g^{-1}), quercetin equivalent (mg QE g^{-1}) and tannic acid equivalent (mg TAE g^{-1}) for phenolic, flavonoid and tannin content, respectively, with values presented as mean ± standard error. To ensure accurate quantitative assessment of antioxidant activity, various antioxidant assays were performed, namely total antioxidant capacity (TAC; expressed as ascorbic acid equivalent, mg AAE g^{-1}), DPPH radical scavenging, OH radical scavenging, metal chelating, and ferric-reducing antioxidant power (FRAP) assays following protocols given by Prieto *et al.*, 1999, Bozin *et al.*, 2006, Aruoma *et al.*, 1987, Decker and Welch, 1990, and Oyaizu, 1986, in that order. *Salvia rosmarinus* was utilized as a positive control in various assays. Further, the plant powder was subjected to GCMS analysis using a TSQ 8000 high-resolution GCMS instrument from Thermo Fisher Scientific, equipped with the NIST20 library for compound identification by comparing mass spectra with known compounds. For identifying functional groups in the phytochemicals FTIR analysis was done using Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer.

All of the phytochemical and antioxidant activity experiments were conducted in triplicates (n=3), and the resulting data were subjected to statistical analysis using SPSS version 16.0. To assess the normal distribution of the data, the Shapiro-Wilk test was employed. Additionally,

the homogeneity of variances was evaluated using Levene's test. For analyzing the differences among various concentrations, ANOVA (Analysis of Variance) was utilized, followed by Tukey's test ($P \leq 0.05$) to determine significant variations among the groups. Furthermore, a two-sampled *t*-test (independent) ($P \leq 0.05$) was employed to identify statistical differences between the extract and the positive control for various antioxidant activities.

RESULTS AND DISCUSSION

Qualitative and quantitative phytochemical analysis

The qualitative analysis of aqueous extract of *B. odora* showed the presence of saponins, terpenoids, coumarins, flavonoids, tannins, and carbohydrates (Table 1). These findings suggest the potential bioactive nature of this plant as these phytochemicals are well-recognized for their diverse therapeutic properties (Pakkirisamy *et al.*, 2017). Building upon the preliminary phytochemical screening, quantitative assessments of phenolics, flavonoids, and tannins were conducted. These compounds are subcategories of polyphenols (Prabhu *et al.*, 2021) that contribute to the diverse pharmacological properties exhibited by plants (Leisegang, 2021). The concentration-dependent TPC was measured in aqueous extracts of *B. odora* which ranged between 0.70±0.290 to 5.97±0.034 mg FAE g^{-1}, across concentrations ranging from 0.0625 to 1%. The statistical analysis revealed a significant difference among all the selected concentrations at $P \leq 0.05$ (Fig. 1). Similarly, TFC in different concentrations of *B. odora* aqueous extracts (0.0625, 0.125, 0.25, 0.5, and 1%) was measured as 0.08±0.001, 0.10±0.003, 0.13±0.010, 0.22±0.002, and 0.34±0.001 mg QE g^{-1}, respectively. TFC was concentration-dependent, showing a significant increase at 0.25, 0.5, and 1% concentrations at $P \leq 0.05$ (Fig. 1). Likewise, TTC varied at different concentrations (0.0625, 0.125, 0.25, 0.5, and 1%) and was found to be 1.58±0.69, 6.16±1.79, 20.83±53.85, 44.35±3.16, and 121.53±14.83 mg TAE g^{-1}, respectively. The statistical analysis showed that lower concentrations (0.625-0.25%) exhibited statistically insignificant effects, however, at higher concentrations (0.5 and 1%), it was significant with $P \leq 0.05$ (Fig. 1).

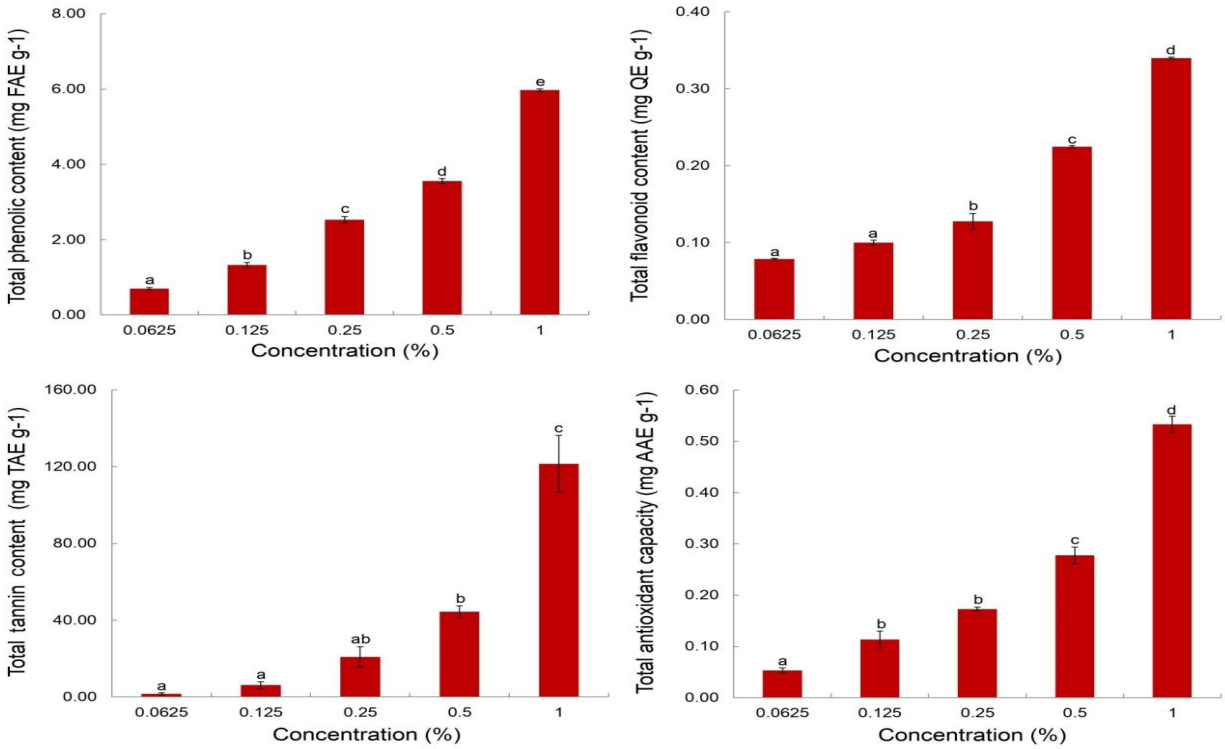

Fig. 1: Total phenolic, flavonoid, and tannin content as well as the total antioxidant capacity of aqueous extract of *Biebersteinia odora* at various concentrations. Data is expressed as mean ±SE of three replicates. Different alphabets represent significant difference among concentration groups at $P \leq 0.05$ applying post-hoc Tukey's test

Table 1: Preliminary phytochemical screening of the aqueous extract of *Biebersteinia odora*

Phytochemical compounds	Presence/absence
Saponins	+
Terpenoids	+
Phlobatannins	–
Glycosides	–
Coumarins	+
Proteins	–
Anthocyanins	–
Flavonoids	+
Anthraquinones	–
Tannins	+
Carbohydrates	+

+ indicates presence; – indicates absence

The quantitative analysis of *B. odora*'s aqueous extract demonstrated concentration-dependent variations in the TPC, TFC, and TTC. The study aligns with findings from similar studies on *B. multifida*, where the extract of different plant parts showed significant antioxidant activities owing to the presence of phenolic and flavonoids (Nabavi *et al.*, 2010). Likewise, the methanolic extract of the leaves of *Geranium robertianum* L., revealed a high amount of phenolics, flavonoids, and condensed tannins which contributed to the plant's antioxidant potential (Jemia *et al.*, 2013).

In-vitro antioxidant activity

The polyphenolic compounds in plants function as antioxidants to reduce the harmful effects of free radicals which are unavoidably created even under normal metabolic settings; as a result, they exhibit a variety of biological properties (Boroja *et al.*, 2018). The presence of polyphenolic compounds in *B. odora* substantiates its antioxidant potential, which is further validated *via* multiple antioxidant assays such as TAC, DPPH radical scavenging assay, OH radical scavenging assay, metal chelating assay, and FRAP. The TAC at different concentrations, i.e., 0.0625, 0.125, 0.25, 0.5, and 1%, was 0.05±0.005, 0.11±0.016, 0.17±0.004, 0.28±0.016, and 0.53±0.016 mg AAE g^{-1}, respectively (Fig. 1). The TAC exhibited a concentration-dependent increase, with statistically significant differences observed at concentrations 0.0625, 0.5 and 1% ($P \leq 0.05$), highlighting the extract's ability to neutralize free radicals. Raeesi *et al.* (2019) evaluated the gastro-protective effect of the methanolic extract of *B. multifida*'s root against the gastric ulcer in rats. The study showed significantly high levels of TAC ascertaining the extract's ability to neutralize free radicals and the role of antioxidant capacity in the gastro protective effects.

The aqueous extract of *B. odora* was also used to determine its antioxidant potential *via* its ability to scavenge DPPH radicals and (Fig. 2). The DPPH radical scavenging activity ranged between 4.89–89.59% whereas that of the positive control ranged between 28.69–97.98%. A significant difference was observed among all the concentrations at $P \leq 0.05$, and the DPPH scavenging activity of the extract was also found to be statistically different from that of the positive control. Similar DPPH scavenging activity was also reported in *G. macrorrhizum* L., where DPPH scavenging activity of methanolic extract of leaves was reported to be 91.7% (Miliauskas *et al.*, 2014). A concentration-dependent pattern was observed in the scavenging power of the OH radical of the aqueous extract of *B. odora*. The OH radical scavenging activity at different concentrations ranged between 13.85–67.57 and 33.96–92.97% for the extract and positive control, respectively (Fig. 2). The statistical analysis showed that concentrations 0.625, 0.125, and 0.25% showed statistically similar effects, as were concentrations 0.25 and 0.5%, whereas, there was a significant difference at 1% concentration with $P \leq 0.05$ (Fig. 2). At all the concentrations, the activity of OH radical scavenging of the extract was also found to be statistically different from that of positive control (Fig. 2).Similar finding was observed in the solvent extract of *Pouzolzia zeylanica* (L.) Benn., a medicinal plant, where the extract exhibited a strong concentration-dependent hydroxyl radical scavenging activity (Li *et al.*, 2011). Metal chelating activity ranged between 11.75–74.75% for the extract and 24.33–84.55% for the positive control (Fig. 2). Statistical analysis showed a significant difference at all the concentrations with $P \leq 0.05$ (Fig. 2) and there was a statistical difference found in the metal chelating activity of the extract and positive control at all the concentrations (Fig. 2). The FRAP of the extract and the positive control was 49.93 and 69.37% at 1% concentration, respectively (Fig. 2). It was

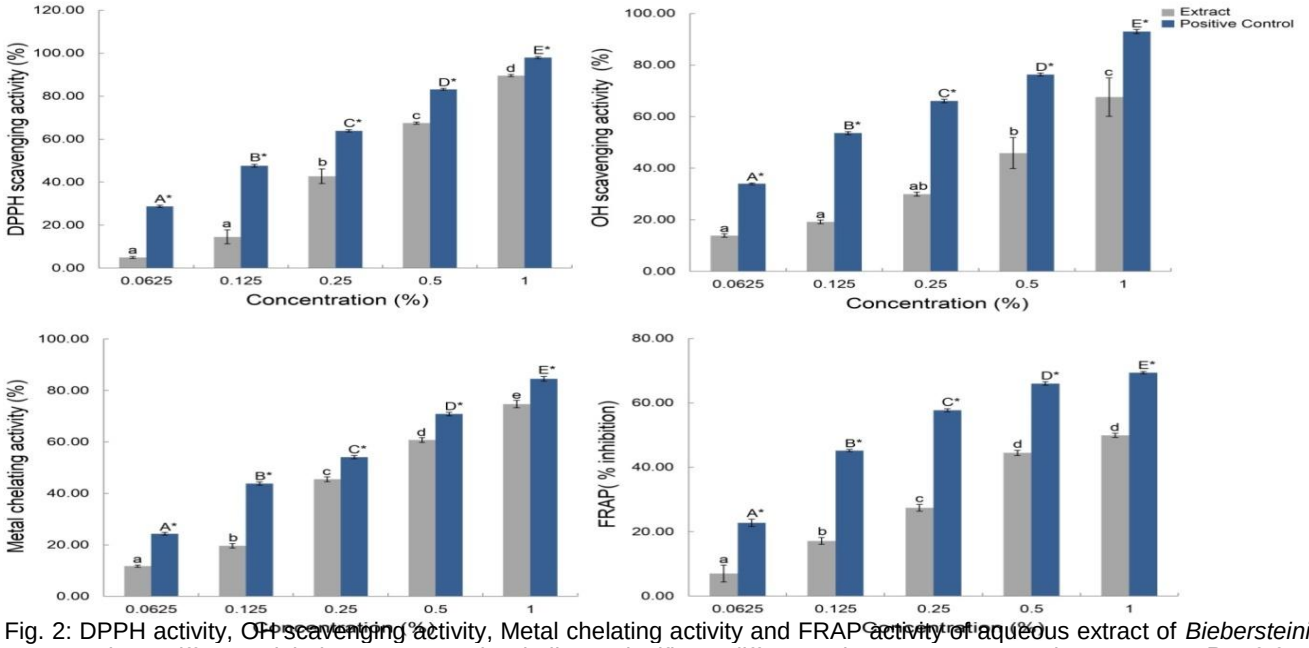

Fig. 2: DPPH activity, OH scavenging activity, Metal chelating activity and FRAP activity of aqueous extract of *Biebersteinia odora*. Different alphabets were used to indicate significant difference between concentration groups at P ≤ 0.05. * represents significant difference among the extract and positive control at a particular concentration at P ≤ 0.05 using two-sampled *t*-test (independent)

statistically significant at concentrations 0.625, 0.125, and 0.25% with P ≤ 0.05, whereas there was no significant difference at 0.5 and 1% (Fig. 2). There was a statistical difference found in the % inhibition of FRAP of the extract and positive control at all the concentrations (Fig. 2).These findings align with a previous study on *B. multifida*'s plant powder extract where a statistically significant dose-dependent increase in the metal chelation and ferric reduction power were observed (Nabavi *et al.*, 2010). Overall results suggest that the aqueous extract of *B. odora* possesses a significant antioxidant potential, emphasizing its application in combating oxidative stress and related diseases and possible therapeutic potential.

Table 2: Phytocomponents identified in the plant powder of *Biebersteinia odora* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis

RT	Peak Area	Area %	Compound	Molecular Formula	Molecular Weight
1.73	26939338.14	6.67	Strychane ,1-acetyl-20α- hydroxy-16-methylene	$C_{21}H_{26}N_2O_2$	338.4
2.1	31329358.3	7.76	Propanal, 2-methyl-	C_4H_8O	72.1
2.81	56008059.81	13.86	Butanal, 2-methyl-	$C_5H_{10}O$	86.1
3.99	37364931.2	9.25	1,3,5-Cycloheptatriene	C_7H_8	92.1
8.58	13000322.29	3.22	Undecane	$C_{11}H_{24}$	156.3
8.67	9957976.48	2.46	Undecane, 3,7-dimethyl-	$C_{13}H_{28}$	184.3
9.29	13320972.59	3.30	Undecane, 4,7-dimethyl-	$C_{13}H_{28}$	184.3
9.81	6819679.43	1.69	Decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	370.7
10.49	6997981.78	1.73	(-)-Borneol	$C_{10}H_{18}O$	154.2
11.94	10223642.82	2.53	2,7,10-Trimethyldodecane	$C_{15}H_{32}$	212.4
12.3	7560689.93	1.87	Dodecamethylcyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	444.9
12.59	8824790.57	2.18	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.4
13.36	5807295.41	1.44	α –Ylangene	$C_{15}H_{24}$	204.3
13.45	6043819.1	1.50	α –Copaene	$C_{15}H_{24}$	204.3
14.06	12715975.09	3.15	Isocaryophillene	$C_{15}H_{24}$	204.3
14.57	35267702.37	8.73	Caryophyllene oxide	$C_{15}H_{24}O$	220.3
15.27	7810617.52	1.93	Muurolene	$C_{15}H_{24}$	204.3
16.49	8748285.57	2.17	Lanceol, cis	$C_{15}H_{24}O$	220.3
16.98	53970032.57	13.36	α-Bisabolol oxide B	$C_{15}H_{26}O_2$	238.3
25.93	33070440.98	8.19	1-Monolinolein, 2TMS derivative	$C_{27}H_{54}O_4Si_2$	498.9
26.64	12205725.85	3.02	(5β)Pregnane-3,20β-diol, 14α,18α -[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	489.6

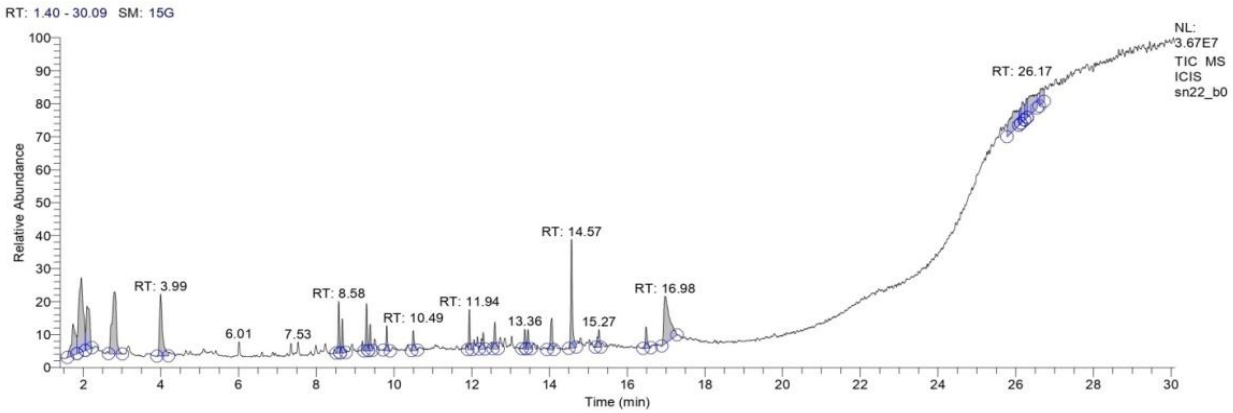

Fig. 3: GC-MS chromatogram of *Biebersteinia odora*

GC-MS (Gas Chromatography- Mass Spectrometry)

A total of twenty-one compounds were putatively identified in the plant powder by GCMS analysis (Fig. 3). These compounds along with their retention time (RT), peak area, area percent, molecular formula, and molecular mass are presented in Table 2. Among these, the prominent compound identified was butanal-2-methyl, constituting 13.86% of the total compounds detected. It is a volatile compound found in plants like *Coffea arabica* L. (Stoffelsma *et al.*, 1968) and *Carica papaya* L. (MacLeod and Pieris, 1983) and known for its aroma and flavor-enhancing properties, often utilized in the food and fragrance industries. The second major compound was α-bisabolol oxide B, comprising 13.36%, an essential oil component found in *Matricaria chamomilla* L. (Ghasemi *et al.*, 2016) and possess anti-inflammatory, anti-irritant, and antibacterial, properties and valuable key component in cosmetics and skincare products (Kamatou and Viljoen, 2010). Other than these, the GCMS chromatogram reported numerous pharmaceutically active compounds in *B. odora* such as caryophyllene oxide, isocaryophillene, lanceol, cis (-)-borneol, α-copaene, known to possess antibacterial, anti-inflammatory, analgesic and anticancer properties (Tung *et al.*, 2008; Karakaya *et al.*, 2020; Di Sotto *et al.*, 2022; Ma *et al.*, 2023). These compounds not only contribute to the chemical complexity of *B. odora* but also hold promise for various applications in pharmaceuticals, cosmetics, and the food industry.

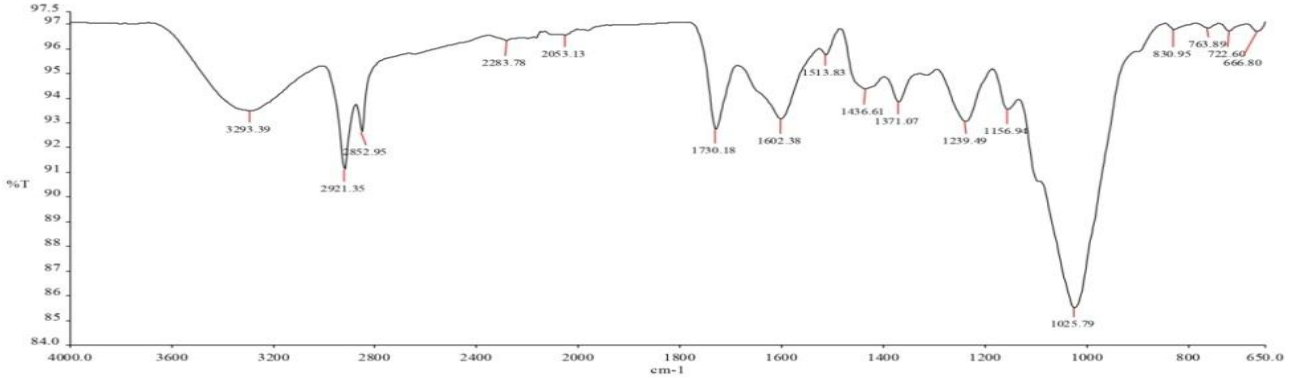

Fig.4: FTIR spectra of *Biebersteinia odora* representing the peak values

FTIR (Fourier-transform infrared spectroscopy)

The detection of specific functional groups is crucial for understanding the chemical composition and potential bioactive properties of the plant. FTIR analysis conducted on the plant powder of *B. odora* has provided valuable insights into the functional groups present. The spectrum showed the presence of various functional groups. The peak values along with the probable functional groups are given in Table 3. The functional group region of the chromatogram showed the presence of alcohol,

Table 3: Fourier-transform infrared spectroscopy (FTIR) analysis representing peak values, bonding, functional groups, and their strength in *Biebersteinia odora*

Peak value	Bonding	Functional group	Strength
3293.39	O-H stretching	Alcohol	Strong
2921.35	C-H stretching	Alkane	Medium
2852.95	C-H stretching	Alkane	Medium
2283.78	O=C=O stretching	Carbondioxide	Strong
2053.13	N=C=S stretching	Isothiocyanate	Strong
1730.18	C=O stretching	Aldehyde	Strong
1602.38	C=C stretching	α,β-unsaturated ketone	Strong
1513.83	N-O stretching	Nitro compound	Strong
1436.61	O-H bending	Carboxylic acid	Medium
1371.07	S=O stretching	Sulphonate	Strong
1239.49	C-O stretching	Alkyl & aryl ether	Strong
1156.94	C-O stretching	Tertiary alcohol	Strong
1025.79	C-N stretching	Amines	Medium
830.95	C=C bending	Alkene	Medium
763.89	C-H bending	1,2-disubstituted	Medium
722.60	C=C bending	Benzene	Strong
666.80	C=C bending/ C-Br stretching	Alkene/ Halo compounds	Strong

alkane, carbon dioxide, isothiocyanate, aldehyde, ketone, and nitro compound, while, the fingerprint region showed the presence of carboxylic acid, sulfonate, alkyl & aryl ether, alcohol, amines, alkene, 1,2-disubstituted, and halogenated compounds (Fig. 4). The identified functional groups reveal a diverse array of chemical constituents within *B. odora*. Specifically, the presence of the OH functional group, indicated by the peak at 3293.39 cm^{-1}, suggests the existence of compounds like polyphenols and such compounds are associated with antioxidant properties (Felhi *et al.*, 2017) and have already been identified as an important functional group in the solvent extract of seeds and fruit peel of *Ecballium elaterium* (L.) A.Rich. (Felhi *et al.*, 2017). Overall, the FTIR analysis underscores the diverse chemical nature of *B. odora*, with the presence of functional groups commonly associated with bioactive compounds in various plants. These findings provide a foundation for future investigations into the potential health benefits and applications of this unique plant extract.

In the aqueous extracts of *B. odora* aerial parts, saponins, terpenoids, coumarins, flavonoids, tannins, and carbohydrates were found to be the main phytochemical components. The quantitative investigation showed that the plant contains a significant amount of phenolics, flavonoids, and tannins. Further, the notable dose-dependent antioxidant potential of *B. odora* was revealed by several antioxidant tests, including TAC, DPPH radical scavenging assay, OH radical scavenging assay, metal chelating assay, and FRAP. The reported effects could be caused by a variety of bioactive substances discovered by GCMS. These results support the traditional therapeutic use of *B. odora*, leading to the conclusion that *B. odora* is a valuable source for the identification of novel bioactive compounds with pharmacological significance.

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