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PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM *PINDA CONCANENSIS*

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Keywords:

Apiaceae, *Pinda concanensis*, Phytochemicals, GC-MS analysis

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ABSTRACT: The plant *Pinda concanensis* belongs to family apiaceae. It was native to the Western Ghats of India, but it is now becoming rare as a result of widespread usage of the seeds for their therapeutic properties. It is an annual herb with tuberous roots, which are eaten raw by local folklore. Apiaceae is the well-known aromatic plant family having beneficial uses in home remedies, abundance of phytochemicals, used in pharmaceuticals and cosmetics. The plant *Pinda concanensis* showed presence of phenolic, flavonoid, alkaloid, terpenoid and saponin content in each plant parts with different solvents of extraction. The methanolic extract of *Pinda concanensis* of different parts showed highest phytocompounds which are significant $P<0.005$.The total phenolic content was highest in dry methanolic stem while total flavonoid content in fresh stem. But total alkaloid, total terpenoid and total saponin was more in methanolic extract of dry root. This is the first report on GCMS analysis of whole plant extract of *Pinda concanensis*, in which twenty-two different compounds are found. The major bioactive compounds are reported are Methoxsalen (11.95%), Ficusin (6.69%), Isopsoralen (1.98%), Phellopterin (1.74%), Cyclohexane,1,2,3,5-tetraisopropyl (5%), Octanoic acid, hexyl ester (4.87%), Hexanoic acid, hexyl ester (2.33%). The chemo profiling of this plant, reveals that the plant parts are rich source of biologically active metabolites and can be considered to treat Psoriasis, Cancer and diabetic like measure diseases thus increasing residence time of drug with better patient compliance.

INTRODUCTION: In traditional medicine, medicinal plants are considered to be the primary source of drug therapy [15]. Thousands of Ayurvedic and household formulations are available in India to address a variety of conditions, including anxiety, melancholy, arthritis, high blood pressure, hormonal imbalances, insomnia, migraines, skin problems, and others.

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The medicinal property of a plant is determined by its physiologically active biochemical compounds, which have an almost unlimited ability to synthesise secondary metabolites found in plant sections such as leaves, fruits, buds, stem, flowers, bark, roots, and so on [13].

Many phytochemicals are used by various physicians in their practices and consumed by people under medical supervision [41]. The main factors influencing the ongoing use of herbal remedies are their efficacy, accessibility, affordability, and lack of or minimal toxic properties [1]. It has an effect on the body that is comparable to that of pharmaceutical medicines, and it can initiate self-healing.

It is important to investigate the therapeutic benefits of the traditional Indian medicinal plants because many phytochemicals with pharmacological action have been isolated from several of these plants [19]. Many instrumental techniques are available to classify and separation of phytocompounds like LCMS, HPLC and GCMS etc. Apart from GCMS is the most precise method for separating and identifying the numerous structurally complex components that are present in plant extracts.

The Family Apiaceae originates all throughout the planet, chiefly at high altitudes in the tropics and northern temperate zones. The primary similarities amongst Apiaceae species include their aromatic herbaceous nature, alternate leaves with sheathing bases, hollow stems, small flowers, inflorescences prescribed in simple or complex umbels, and indehiscent fruits which showed mericarp or seeds, the oil ducts found in seed [7] and in leaves (Jillian Stansbury, 2016). Since ancient times, spices and herbs have been used in cuisine as food preservatives as well as flavouring enhancers. In folk medicine, they have also been employed [16].

Pinda concanensis is a monotypic genus from family apiaceae. The synonyms of Pinda are *Heracleum concanensis*, *Heracleum pinda*, and *Heracleum grandiflorum*. According to (Mbosso *et al*., 2016) it becomes in low risk [26]. While Deshmukh B. S. (2010) the *Pinda concanensis* was endemic to Western Ghats of India and now becoming rare because of seeds were used heavily for its medicinal value [8]. Leaves of *Pinda* have 2-3 pinnate, flowers are white in colour, ebracteate having exterior petal of marginal flower grossly dilated. Fruits are dorsally compressed with projecting fruit ribs but not winged and vittae extending to base of fruit. These are key characters of *Pinda concanensis*. There is little found in phytochemical analysis in *Pinda concanensis*, therefore our research gives the idea about the presence of phytochemicals and GCMS analysis characterizes the bioactive compound which present in methanolic extract of *Pinda concanensis.*

MATERIAL AND METHODS:

Collection and Conservation of Plant *Pinda concanensis*: The Plant *Pinda concanensis* was collected from different localities of Maharashtra. (Kas Plateau, Satara). The plant was identified, germplasm of these plants was maintained in the Botanical Garden of Botany Department, Shivaji University, Kolhapur for the further study.

Preparation of Herbarium Specimen: Herbarium for voucher specimens was made from compiled plant material that has a proper phenological state. The whole plant was dipped in 30-40% formalin for one to two days and pressed on blotting paper under the wooden press. The specimens were dried and the paper was changed afterward using the proper method. The dried specimens were poisoned, mounted, and labeled on the herbarium sheet by the standard method (Forman and Bridson, 1991). *Pinda concanensis* species herbarium specimen were deposited in the herbarium of the Department of Botany, Shivaji University, Kolhapur.

Chemo-profiling of *Pinda concanensis*: *Pinda concanensis* was analyzed for the biochemical which were newly reported in that plant like Total Phenolics, Total Flavonoids, Total Alkaloids, Total Saponins, and Total Terpenoids on the basis of their fresh and dry plant parts. (i. e. Root, Stem, Leaf and Seed).

Preparation of Extract for Biochemical Analysis: The plant material was dried, then pulverized and was kept in dry until analyzed. The fresh and dry plant material extract was prepared by using two solvents; methanol and distilled water. The different parts of plant (25 gm) were ground using laboratory grinder. The extracts were then filtered through Whatman filter paper No.1 by using Buchner's funnel and final volume was adjusted to 100 ml with respective solvents. For each plant part same extraction procedure with different solvents was adopted. The concentrate 25% extract were prepared from that out from that I was used 10% extract for my research work which was prepared by using that concentrate extract. All the plant extracts (10%) were stored in refrigerator at 4^{o}C and were used for further analysis.

Principal Quantitative Analysis of Phytoconstituent: The prepared 10% stock of each solvent extract was used directly for quantitative analysis or diluted as per requirement. The following phytochemical assays were performed to

screen *Pinda concanensis* species for the quantification of the major phytochemical compounds for specific bioactivity.

Estimation of Total Phenolic Content: Total phenolic contents (TPC) of the plant extracts were assessed using Folin - Ciocalteu method [40]. For to estimated total phenolic content, a 96-well microtiter plate method was used. The reaction mixture was prepared by mixing 50 µl of distilled water in each well, an aliquot of extracts 12.5 µl with 12.5 µl Folin - Ciocalteu reagent, then incubate for 10 minutes at room temperature 37^0C, after incubation add 0.125 µl of saturated sodium carbonate (7% Na_2CO_3) and 100 µl in this mixture. Then again incubated for 90 min at room temperature in dark 37°C. The absorbance was measured at 760 nm in 96 well microplate (Multiskan sky spectrophotometer, Thermoscientific). The assays were prepared in triplicates for each analysis and the mean values of absorbance were obtained. The readings were compared with a standard phenolic compound i.e. Tannic acid and were expressed as milligram Tannic acid equivalents per gram Fresh weight/Dry weight (mg TAE/ of DW or FW) in a result Total phenolic content were calculated by using calibration curve of standard tannic acid.

Estimation of Total Flavonoid Content: In this the Total flavonoids content (TFC) of the different plant parts were analyzed by using modified colorimetric method [20]. The reaction mixture was prepared by mixing 150 µl 2% methanolic $AlCl_3$ with 150 µl plant extract. Incubation of 10 minutes at room temperature was done. After incubation the absorbance was measured at 368 nm in 96 well microplate (Multiskan sky spectrophotometer, Thermoscientific). The assays were prepared in triplicates for each analysis and the mean values of absorbance were obtained. The calibration curve of quercetin was used for to calculate total phenolic content. The results were expressed as milligram Quercetin equivalents per gram fresh weigh/dry weight (mg QE/g of DW or FW).

Estimation of Total Alkaloid Content: Total alkaloid content (TAC) of the extracts was assessed using 1, 10-phenanthroline method described by [37]. The assay mixture was prepared by using100 µl plant extract, 100 µl 1,10-phenanthroline and 100 µl FeCl3 in 0.5M HCl, make a total volume 1 ml by using distilled water and incubated for 30 minutes in water bath maintained at 70±2° C (Till orange colour appear). Above reaction mixture, excluding 1% plant extract, substituted by distilled water was served as a blank. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained.

The absorbance was measured at 510 nm in 96 well microplate against reagent blank (Multiskan sky spectrophotometer, Thermoscientific). The OD measurements were compared to standard curve of colchicine (a standard alkaloid) and results expressed as milligrams Colchicine Equivalent (CE) per gram of fresh weight (FW) or dry weight (DW) of respective plant parts of *Pinda concanensis*.

Estimation of Total Terpenoid Content: Total triterpenoid content was determined by colorimetry using Chang *et al*, (2011) method with slight, modification. 25 µl of Plant extract was mixed with 37.5 µl vanillin-glacial acetic acid solution (5% w/v) and 125 µl of Perchloric acid. Then samples were heated for 45 min at 60^0C and cooled on ice bath. Absorbance was measured at 548nm wavelength after the addition of 562.5 µl of glacial acetic acid, using 96 well plate reader (Multiskan sky spectrophotometer, Thermoscientific). The total terpenoid content was calculated from the calibration curve and the results were expressed as mg of Ursolic acid equivalent per gm fresh or dry weight.

Estimation of Total Saponin Content: Saponin content in *Pinda concanensis* was determined according Hiai *et al*., (1975) to the method [12] with slight modification. Total Saponin also known as vanillin-sulfuric acid assay. In this, reaction mixture consists of 50 µl of plant extract, 50 µl of 8% vanillin to which 500 µl of 72% H_2SO_4 was added. Reaction mixture was incubated for 10 min at 60°C in water bath. Allowed to cool and absorbance was measured at 544 nm (Multiskan sky spectrophotometer, Thermoscientific) wavelength. The total saponin content was calculated from the calibration curve and the results were expressed as mg of Diosgenin equivalent per gm fresh or dry weight.

Statistic: All assays performed in triplicate the analysis of result was done by using Graph pad prism to identify significance value which was $p<0.005$.

Phytochemical Profiling of Plant Soxhlet Extract by using GCMS Analysis:

Sample Preparation: The seeds powdered (2gm) extract of *Pinda concanensis* were prepared in methanol by using Soxhlet extraction method. After extraction excess methanol was evaporated on a water bath. Remaining residue stored at 4 °C until further analysis.

GC-MS Analysis: The samples were run, data was studied and interpretative results of the different chromatogram peaks were attempted from the GC-MS program. The identification of the compounds was made by observing their mass spectra and retention indices with the NIST 14 library of compounds using a search engine Shimadzu (SH-Rxi™-5Sil MS) mass spectrophotometer. The separations were achieved by capillary column, Phenomenex ZB5 (30m ×0.25mm×film thickness0.25µm). The column temperature was kept at 40^0 C for 1 minute, injector temperature-250^0C holding time 5 minute and split ratio was adjusted at 1:70. The flow rate of helium as carrier gas was 1mL/min. The MS were taken at 70eV electron ionization, ion source temperature 200°C. Interpretation of mass spectroscopy (GC-MS) was conducted using the data base of National Institute standard And Technology (NIST). The spectrum of unknown component was compared with the spectrum of known component stored in the NIST library.

RESULT AND DISCUSSION: Phytochemicals are the chemicals produced by plants, specially the secondary metabolites, synthesized as a measure for self-defence against insects, pests, pathogens, herbivores, ultraviolet exposure and environmental hazards. There are no previous findings on quantitative phytochemical examination of *Pinda concanensis*, as this is our first piece of report on quantification of phytochemicals. So, in present investigation total phenolic, flavonoid, alkaloid, terpenoid, and saponin contents were discovered quantitatively and by through GC-MSMS analysis. In Phytochemicals phenolic compounds are the largest group which were widely distributed in various higher Plants like vegetables, fruits grains and nuts. Which performed very important role in various physiological processes like plant quality, colouring, flavour, and stress resistance. In earlier research field anti-inflammatory, antibacterial, anticarcinogenic, and antioxidant properties of phenolic compounds have attracted a lot of attention from researchers. The concentration of total phenolic content in both fresh and dry extracts of stem root and leaf of *Pinda concanensis* were represented in **Table 1** and **Fig. 1** respectively.

The methanolic dry stem showed highest total phenolic content (151.39±0.033 mg TAE /gm of dry weight), which was followed by methanolic dry root (114.42±0.010 mg TAE /gm of dry weight).But dry aqueous extract of stem showed (97.76±0.003 mg TAE /gm of dry weight). Whereas both fresh and dry aqueous extract of leaf showed (67.15±0.026 mg TAE/gm of fresh weight and 60.79±0.011 mg TAE/gm of dry weight) respectively which was highest than methanolic extracts.

The total phenolic content of methanolic extract of leaf of *Heracleum lasiopetalum* Bois was (120±2.12 mg TAE/ of dry weight) which was highest than our findings concern with leaf as well as the plant *Echinophora platyloba* DC. Exhibit (74±4.21 mg TAE/ of dry weight)in methanolic stem which were lower than our findings in *Pinda concanensis* [32]. But in *Coriandrum sativum* the methanolic extract of dry stem showed 47.32 mg GAE/gm of dry weight as well as fresh aqueous extract of root showed highest phenolic content 62.6 mg GAE/gm of fresh weight [35].

While, the dry aqueous extract of *Pinda concanensis*is highest (2.317±0.33 mg of GAE/gm of dry weight) than methanolic extract (0.787±0.02 mg of GAE/gm of dry weight) [36]. According to Magulska and Wesolowski (2018) that the caraway seed extract showed 8.7–17.3 mg GA/g. Secondary metabolites produced by complete plants are called polyphenols. These substances include, among others, phenols, phenolic acids, quinones, flavonoids, tannins, and phenylpropanoids. In recent years importance of polyphenolic compounds in human health is increases which act as antioxidant, anti-mutagenic, and scavenging activity on free radicals and prevention of

pathologies such as cancer and cardiovascular heart diseases [29]. Polyphenolic compounds have been found to defend erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants [17, 3, 4, 39].

TABLE 1: TOTAL PHENOLIC CONTENTS *PINDA CONCANENSIS*

Solvent	Plant Parts	TPC (mg/gm) TAE
Methanol	Dry Root	114.42±0.010
	Dry Stem	151.39±0.033
	Dry leaf	52.00±0.003
	Fresh Root	74.73±0.001
	Fresh Stem	67.15±0.006
	Fresh leaf	51.70±0.003
Aqueous	Dry Root	12.91±0.006
	Dry Stem	97.76±0.003
	Dry leaf	60.79±0.011
	Fresh Root	38.67±0.002
	Fresh Stem	26.55±0.008
	Fresh leaf	67.15±0.026

Data are expressed in Tannic acid equivalent (TAE) per gram of plant material (Fresh or Dry, R^2=0.9738). P value is<0.001****Calculated by using one-way ANOVA. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root.

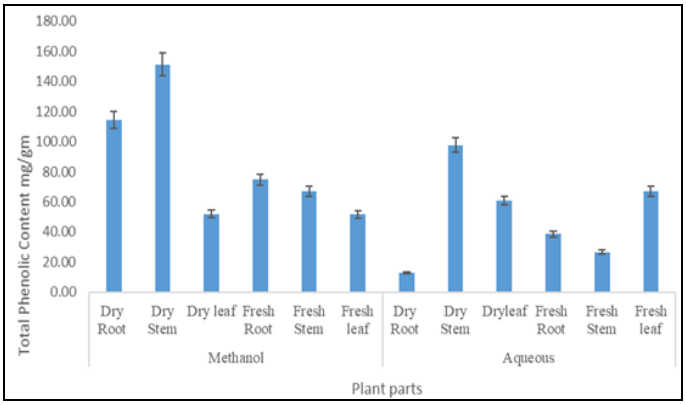

FIG. 1: GRAPHICAL REPRESENTATION OF TOTAL PHENOLIC CONTENTS OF *PINDA CONCANENSIS*. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root

Flavonoids are the most common, widely distributed and a major colouring component of flowering plant are found in all plant food. The total flavonoid content in both extract methanol and aqueous of root, stem and leaf of *Pinda concanensis* were depicted in **Table 2** and **Fig. 2** respectively. All methanolic extracts of *Pinda concanensis* displayed high amount of total flavonoid content than aqueous extract, apart from that the quantity of total flavonoids are highly quantified in methanolic extract of fresh stem (43.06±0.088 mg QE / gm of fresh weight), which is followed by methanolic extract of dry root (29.35±0.040 mg QE / gm of dry weight). Methanolic extract of fresh leaf have lowest content of total flavonoid. (14.83±0.011 mg QE / gm of fresh weight) Over all view, We report here that the total flavonoid content is highest in methanolic extracts than aqueous extracts of *Pinda concanensis*, according to Shimpale *et al*., (2018) the flavonoid content is highest in methanolic extract (6.14±0.28 mg of QE/gm of dry weight) than aqueous extract (3.58±0.19 mg of QE/gm of dry weight) [36] which are somewhat similar with our findings. Flavonoids are abundantly found in human diet regularly, generally these are nontoxic and a diverse range of biological activities, like modulation of steroid hormones, inhibition of proliferation, anticarcinogenic and antioxidative.

TABLE 2: TOTAL FLAVONOID CONTENTS *PINDA CONCANENSIS*

Solvent	Plant Parts	TFC (mg/gm) QE
Methanol	Dry Root	29.35±0.040
	Dry Stem	20.33±0.029
	Dry leaf	27.42±0.007
	Fresh Root	15.22±0.018
	Fresh Stem	43.06±0.088
	Fresh leaf	14.83±0.011
Aqueous	Dry Root	7.84±0.006
	Dry Stem	6.08±0.003
	Dry leaf	18.46±0.021
	Fresh Root	9.86±.049
	Fresh Stem	13.17±0.006
	Fresh leaf	17.24±0.057

Data are expressed in Quercetin equivalent (QE) per gram of plant material (Fresh or Dry, R^2= 0.9849). P value is<0.001****Calculated by using one-way ANOVA. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root

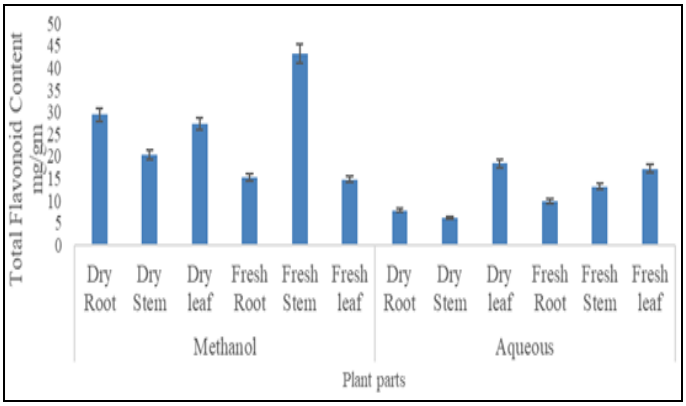

FIG. 2: GRAPHICAL REPRESENTATION OF TOTAL FLAVONOID CONTENTS OF *PINDA CONCANENSIS*. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root

Alkaloids comprise the largest class of secondary plant substances (Harborne, 2012). They are supposed to function as growth regulators and insect repellents or attractants (Harborne, 2012) [5] reported that alkaloids include organic acid salts, mainly malate, acetate, and citrate, or they may be coupled with other molecules, such tannins, to create other chemicals in plants.

Overall majority of alkaloids display basic characteristics and possess a lipophilic character, keeping it soluble in alcohol and a polar organic solvent. According's to the finding, the methanolic and aqueous extracts of dry root of *Pinda concanensis* showed maximum amount of total alkaloid (192.26±0.018 mg CE/gm of dry weight and 133.70±0.026 mg CE/gm of dry weight) represented in **Table 3 and Fig. 3** respectively.

But methanolic extract of fresh stem (177.41±0.027 mg CE/gm of fresh weight) also showed highest content of total alkaloid than aqueous extract fresh stem, whereas fresh aqueous extracts of root contain least amount (27.79±0.009mg CE/gm of fresh weight).

As well as [36] reported that (16%) total alkaloid was found in methanolic extract of *Pinda concanensis.* The results of clarified that the plant of *Pimpinella armena* and *Pimpinella kotschyana* showed percentage of total alkaloid (22.8% and17.8% respectively) [28].

TABLE 3: TOTAL ALKALOID CONTENTS *PINDA CONCANENSIS*

Solvent	Plant Parts	TAC (mg/gm) CE
Methanol	Dry Root	192.26±0.018
	Dry Stem	53.39±0.010
	Dry leaf	88.24±0.009
	Fresh Root	92.03±0.015
	Fresh Stem	177.41±0.027
	Fresh leaf	67.26±0.018
Aqueous	Dry Root	133.70±0.026
	Dry Stem	35.97±0.005
	Dry leaf	51.58±0.007
	Fresh Root	27.79±0.009
	Fresh Stem	52.79±0.009
	Fresh leaf	85.21±0.038

Data are expressed in Colchicine equivalent (CE) per gram of plant material (Fresh or Dry, R^2= 0.9255). P value is<0.001****Calculated by using one-way ANOVA. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root.

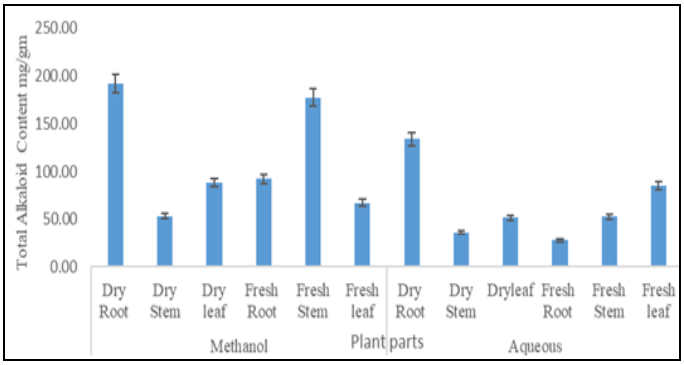

FIG. 3: GRAPHICAL REPRESENTATION OF TOTAL ALKALOID CONTENTS OF *PINDA CONCANENSIS*. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root

Terpenoids constitute one of the largest and structurally diverse groups of naturally occurring compounds in that natural products derived from mevalonic acid (MVA). Terpenoids are widely found in nature which were used in pharmaceutical purposes like making in cosmetics, insecticides, pesticides [38, 2, 22]. In this **Table 4** and **Fig. 4** shown that root extracts of both solvent of *Pinda concanensis* have more terpenoids than other plant parts. While Aqueous extract dry root exhibit high amount of total terpenoid content (141.40 ±0.010 mg UAE/gm of dry weight), which was followed by methanolic dry root extract showed also high amount of total terpenoid (108.68 ±0.015 mg UAE/gm of dry weight). While methanolic extract of dry leaf good amount of terpenoid (47.32 ±0.004 mg UAE/gm of dry weight) whereas aqueous fresh leaf expressed least amount of terpenoid (14.23 ±0.006 mg UAE/gm of dry weight).

TABLE 4: TOTAL TERPENOID CONTENTS *PINDA CONCANENSIS*

Solvent	Plant Parts	TTC (mg/gm) UAE
Methanol	Dry Root	108.68 ±0.015
	Dry Stem	20.15 ±0.003
	Dry leaf	47.32 ±0.004
	Fresh Root	88.73 ±0.038
	Fresh Stem	39.65 ±0.032
	Fresh leaf	19.40 ±0.008
Aqueous	Dry Root	141.40 ±0.010
	Dry Stem	15.48 ±0.005
	Dry leaf	14.90 ±0.007
	Fresh Root	72.07 ±0.004
	Fresh Stem	15.07 ±0.048
	Fresh leaf	14.23 ±0.006

Data are expressed in Ursolic acid equivalent (UAE) per gram of plant material (Fresh or Dry, R^2= 0.9958). P value is<0.001**** Calculated by using one-way ANOVA. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root.

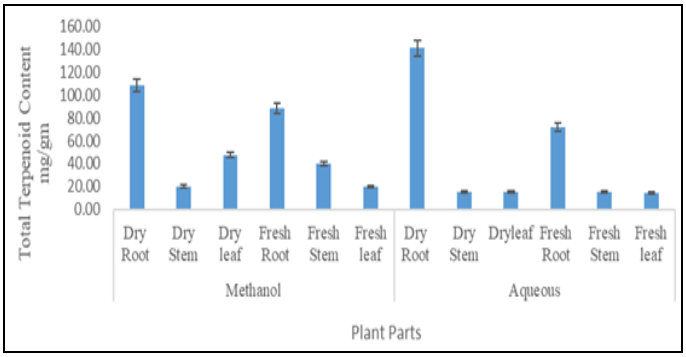

FIG. 4: GRAPHICAL REPRESENTATION OF TOTAL TERPENOID CONTENTS OF *PINDA CONCANENSIS*. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root

The therapeutic potential of phytochemicals is very well recognized. As an example, Saponins are amphiphilic molecules with carbohydrate and either triterpenoid or steroid aglycone moieties that have been shown to have fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant, and immunomodulatory properties (Juang *et al*., 2020). As shown in **Table 5** and **Fig. 5**, total saponin content is highest in dry and fresh methanolic root extract (38.15 ±0.020 mg DE/gm dry weight and 31.37 ±0.001 DE/gm fresh weight).

TABLE 5: TOTAL SAPONIN CONTENTS *PINDA CONCANENSIS*

Solvent	Plant Parts	TSC (mg/gm) DE
Methanol	Dry Root	38.15 ±0.020
	Dry Stem	22.46 ±0.031
	Dry leaf	37.86 ±0.018
	Fresh Root	31.37 ±0.001
	Fresh Stem	36.08 ±0.042
	Fresh leaf	20.39 ±0.034
Aqueous	Dry Root	27.00 ±0.062
	Dry Stem	19.36 ±0.010
	Dry leaf	17.75 ±0.006
	Fresh Root	20.22 ±0.023
	Fresh Stem	14.93 ±0.010
	Fresh leaf	17.69 ±0.015

Data are expressed in Diosgenin equivalent (DE) per gram of plant material (Fresh or Dry, R^2= 0.9146). P value is<0.005**Calculated by using one-way ANOVA. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= Root

In case of leaf, dry methanolic extract showed good content of total saponin (37.86 ±0.018 mg DE/gm dry weight) which was followed by fresh methanolic stem (36.08 ±0.042 mg DE/gm fresh weight). According to Mandal *et al*., 2002 the dry methanolic leaf extract of *Eryngium foetidum* showed highest saponin content (292 mg QSE/g) [21] than *Pinda concanensis*. According to the findings saponins are abundant in root of *Pinda concanensis*. But *Pinda concanensis* exhibited total saponins i.e 19.91% [36].

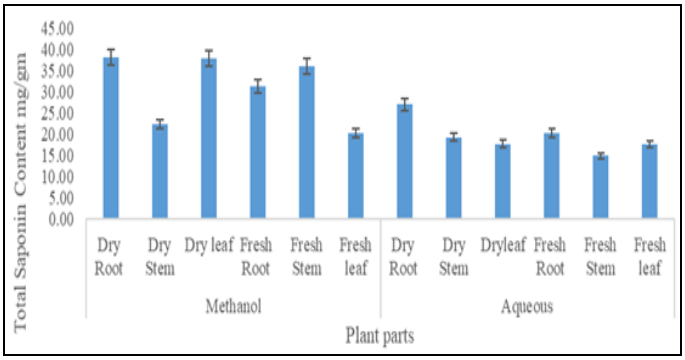

FIG. 5: GRAPHICAL REPRESENTATION OF TOTAL SAPONIN CONTENTS OF *PINDA CONCANENSIS*. F=Fresh, D=Dry, A=Aqueous, M=Methanol, S= Stem, L= Leaf, R= R.

Chemo-profiling of Bioactive Compound by using GCMS Analysis: Gas chromatography mass spectrometry (GC-MS) has established its position as an important technical platform for bioactive compound profiling in both plant and non-plant species over the past few years [9, 19]. A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide, related to the possible chemical components of *Pinda concanensis* extract by using GCMS analysis. In this analysis we got total 22 new bioactive compound that mean first time detected in *Pinda concanensis.* The presence of multiple components with different retention times was confirmed by the GC-MS spectra as shown in **Fig. 6** Interpretation of mass spectroscopy (GC-MS) was conducted using the data base of National Institute Standard and Technology (NIST). The spectrum of unknown component was compared with the spectrum of known component stored in the NIST library in **Table 6**. Many of these compounds are medicinally important which showed biological activity in other members of apiaceae.

Out of detected 22 compounds biologically active compounds were notify in the **Table 6** with its molecular area(%) molecular weight and its reference. The compound Methoxsalen having highest area (11.95%) which was found in high amount in plant extract with molecular weight (216) having anti-diabetic osteoporosis activity (Ham *et al*., 2006).

Ficusin is another compound with high content are (6.69%) which was the coumarin found in *Ficus* sp. Which showed various biological activities like antiplasmodial [27], antioxidant [30], anticancer [23], antimicrobial [23] antiulcer [11], antidiarrhoeal [21], anti-pyretic [33], and gastroprotective [34]. The compound Cyclohexane, 1,2,3,5-tetraisopropyl- having the percentage of area (5%) which showed various biological activities like Antieczematic, Antioxidant, Antimutagenic, Antiseborrheic [17] with molecular weight (252). Pimpinellin is the compound detected in many apiaceae species which was first time recorded in *Pinda concanensis* with percentage area (4.83%) and molecular weight (246) which was again furanocoumarin showed Anticancer and Antiviral activity [14] Hexanoic acid, hexyl ester is the compound used in Flavouring agents which was also exhibit Antidiabetic activity, anticancer activity [44]. Octanoic acid, hexyl ester presented antimicrobial activity [42]. The furanocoumarin Isopsoralen with molecular weight (186) exhibited anti-Psioraisis, used in Vitiligo and Skin disorder [14]. The compound Falcarinol showed cytotoxicity [43] having percentage (1.62%) The compound Phellopterin showed Hepatoprotective Activity and Anti-inflammatory activity [10]. Most of the compound showed Antibacterial, Antioxidant and Anti-inflammatory activity. According to some references some compounds are used in treatment of Vitiligo, Psoriasis. Most of the compounds are coumarins, which is the key compound of family apiaceae.

TABLE 6: ACTIVITY OF BIOACTIVE COMPOUND IDENTIFIED IN METHANOLIC EXTRACT OF *PINDA CONCANENSIS*

Peak No.	R. Time	Area%	Name	Mol. Wt	Biological activity
1	10.127	0.32	Octanoic acid	144	Antimicrobial activity [42]
	13.234	2.33	Hexanoic acid, hexyl ester	200	Flavouring agents Antidiabetic activity, anticancer activity [44]
8	15.734	4.87	Octanoic acid, hexyl ester	228	Antimicrobial activity [42]
11	18.118	1.98	Isopsoralen	186	Psioraisis, Vitiligo and Skin disorder [14]
12	18.750	6.69	Ficusin	186	antiplasmodial [28], antioxidant [31] anticancer [24, 25, 26] antiulcer [11], antidiarrhoeal [21], anti-pyretic [33] and gastroprotective [34]
13	21.031	1.62	Falcarinol	244	Cytotoxic activity[43]
14	21.203	11.95	Methoxsalen	216	Prevent diabetic osteoporosis (Ham *et al*., 2006)
16	21.935	1.74	Phellopterin	300	Hepatoprotective Activity and Anti-inflammatory [10]
17	24.591	4.83	Pimpinellin	246	Anticancer, Antiviral [14]
21	33.300	5.00	Cyclohexane, 1,2,3,5-tetraisopropyl-	252	Antieczematic, Antioxidant ,Antimutagenic, Antiseborrheic [17]

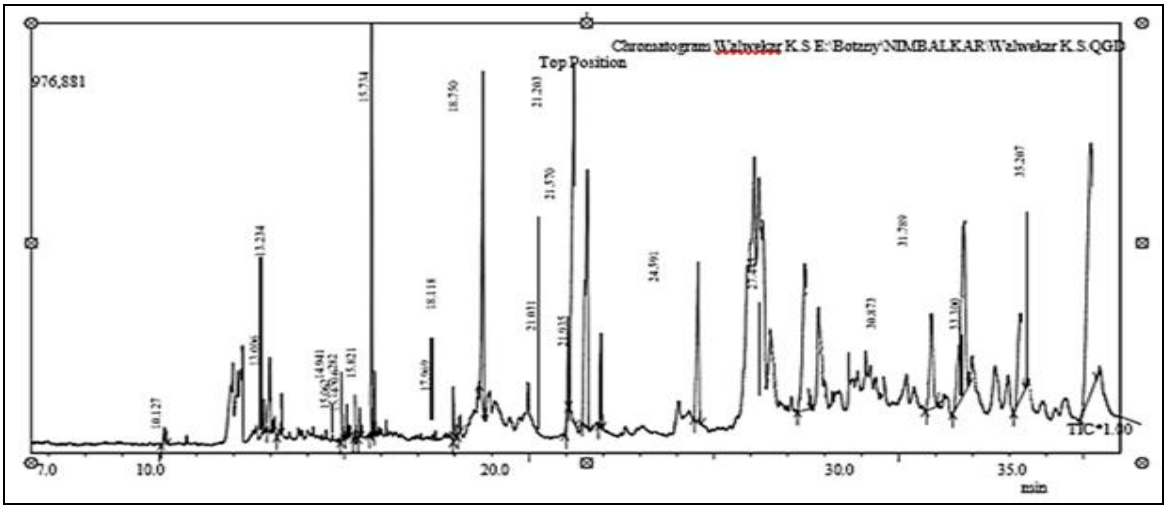

FIG. 6: GCMS CHROMATOGRAM OF METHANOLIC EXTRACT OF *PINDA CONCANENSIS*

CONCLUSION: Thus, the findings of this research suggest that the identified phytochemical compounds are bioactive constituents. The results of these experiments provide the chemical

foundation for the widespread use of this plant as a therapeutic agent for a variety of disorders. The plant *Pinda concanensis* first time evaluate for phytochemical analysis. Which was also used in preparation of herbal alternative for a variety of illnesses such as diabetes, cancer, microbial infections, inflammations, and so on.

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