

Original Research Article

PHYTOCHEMICAL PROFILE AND ANTIOXIDANT POTENTIAL OF A FORMULATED HERBAL PRODUCT FROM *SORGHUM BICOLOR* LINN., *CURCUMA LONGA* LINN., *BRIDELIA FERRUGINEA* BENTH AND HONEY

Abstract

The use of plants in the treatment of human diseases is as old as human itself. Over the years, phytomedicine has gained more attention in both developed and developing countries. This study evaluates the phytochemicals and the antioxidant properties of a formulated herbal product (FHP) made from the leaf sheath of *Sorghum bicolor*, rhizome of *Curcuma longa*, stem bark of *Bridelia ferruginea* and honey. Both the ethanolic and the aqueous extracts of the different components and the mixture were evaluated. The chemical compositions of the extracts were determined via gas chromatography-mass spectrometry (GC-MS) analysis and High Performance Liquid Chromatography (HP-LC) while the antioxidant properties were investigated using the ferric reducing antioxidant power (FRAP). The results of the phytochemical screening of FHP and the different components used for its preparation showed the presence of alkaloids, phenols, saponins, tannins and flavonoids irrespective of the extractant used. However, the values were higher in the aqueous extracts than the ethanolic extracts for most of the phytochemicals. For instance, higher values of alkaloids (9.57 ± 0.01 mg/g) and saponins (7.35 ± 0.01 mg/g) were observed in the aqueous extract of FHP while higher values of phenol (0.98 ± 0.01 mg/g), tannins (0.68 ± 0.00 mg/g) and flavonoids (40.54 ± 0.05 mg/g) were observed in the aqueous extract of turmeric. Concerning antioxidant potential, the highest FRAP power was observed in the stem bark of *B. ferruginea* (1956.05 ± 15.54 mgAAE/g). This was closely followed by the FRAP value observed in *S. bicolor* (1359.89 ± 27.20 mgAAE/g). A total of 8 compounds was identified using High performance Liquid chromatography. These compounds include Gallic acid, Catechin, Ferrulic acid, Rutin, Quercetin, P-coumaric acid, Kampferol and Apigenin. Most of these chemicals are known to possess health beneficial and therapeutic effects. It is conceivable therefore that FHP might exert significant therapeutic effects when used to treat specific debilitating medical conditions.

Key words: Formulated herbal product, phytochemicals, antioxidant property, organic compounds

Significant Statement

The search for new drugs is on the increase because most of the available drugs are becoming non-effective as a result of overuse and drug resistance by most pathogenic microorganisms. Different plants have long been exploited in folklore medicine therefore researching into plants as source of discovery of new drugs has gained so more attention not only in the developing

countries but also in the developed countries. This present study focuses on screening a formulated herbal product (FHP) made from the leaf sheath of *Sorghum bicolor*, rhizome of *Curcuma longa*, stem bark of *Bridelia ferruginea* and honey for phytochemicals and antioxidants. The results of the phytochemical screening of FHP revealed the presence of alkaloids, phenols, saponins, tannins, flavonoids and some organic compounds which are known to possess health and therapeutic effects such as Gallic acid, Catechin, Ferrulic acid, Rutin, Quercetin, P-coumaric acid, Kampferol and Apigenin. It is conceivable therefore that FHP might exert significant therapeutic effects when used to treat specific debilitating medical conditions.

Introduction

Many plants referred to as medicinal plants are used as the raw material for many herbal formulations and supplements. The use of herbal medicines has been on the rise in recent years due to its low price [1]. About 80% of the people living in developed countries use traditional medicine [2]. Plants are used by humans as phytomedicine because of their bioactive constituents [3]. The active constituents of some plants such as flavonoids, polyphenols, alkaloids, terpenoids, saponins, enzymes, and tannins are widely used for prevention and treatment of many diseases. Nowadays, there is a great interest in drugs from natural origin with minimal side effects as against synthetic ones [4, 5, 6].

Sorghum (*Sorghum bicolor* Linn.) is a food grain that is grown across America and Africa. *Sorghum* species are known to have a high content of antioxidants, including simple phenolic acids, as well as polyphenols, particularly 3-deoxyanthocyanidins, such as luteolinidin and apigenidin [7]. The high content of these antioxidant compounds are present in sorghum-based beers and contribute to the inhibition of lipid peroxidation during mashing and boiling. The leaf sheath has a different chemical composition than the leaf blade [8]. The sorghum leaf sheaths have a strong chemoprotective potential and inhibit proliferation of gastrointestinal cancer cell lines, and these effects are partially independent of their antioxidant content [9]. The anticancer properties of sorghum are due in part to the high content of 3-deoxyanthocyanidins [9, 10]. In addition to the high content of anti-inflammatory phenolic compounds, sorghum contains several groups of bioactive compounds with the capacity to induce proinflammatory immune responses. Water-soluble beta-glucans found in sorghum are biologically active and are capable of initiating macrophage activation [11]. According to Ali *et al.* [12], the therapeutic roles reported on sorghum are as a result of anti-inflammatory, anti-carcinogenic [9, 10] antibiotic, antifungal, antiviral, hepatoprotective, anti-ulcer, anti-neoplastic, cholesterol-lowering and digestibility showing properties. Such pharmaceutical functions are allied to the phytochemical contents of the plant such as phytosterols, policosanols, saponins, carotenoids and phenolic compounds, including flavonoids, tannins, and anthocyanins [13]. Different important flavonoids and phenolic acids identified in the leaves extract of sorghum include gallic acid, catechin,

chlorogenic acid, caffeic acid, ellagic acid, rutin, isoquercitrin, quercitrin, quercetin, and kaempferol [14].

Curcuma longa Linn. (Turmeric) is a tropical plant of South, South-eastern Asia, and primarily found grown in tropical regions of Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, Phillipines and Nigeria [15]. Turmeric has healthy influence on digestive system and it also enhances the mucin secretion in the digestive tract. Several actions of turmeric have been identified. It has antibacterial, antihelminthic, anticancer, antiparasitic, antiseptic, anti-oxidative [16], anti-inflammatory, anti-rheumatic, anti-tumour, anti-phlegmatic, antiviral activities. Moreover, it has astringent, aromatic and blood purifying ability. It can also clear skin blemishes and neutralize free radicals. Furthermore, it has hepatoprotective and nephroprotective properties against toxic agents-induced hepato-renal failure [17, 18]. Also, it has protective activity against cytotoxic, teratogenic, and neurodegenerative diseases [5], anticoagulant and possess anti-HIV activity to combat AIDS [3].

Bridelia ferruginea Benth. (Euphorbiaceae) is a plant that is commonly found in Savannah regions [19]. Its bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle “Egun Efu”. It is also reported of having potential for water treatment and chemo preventive potentials [20]. Several phenolic compounds isolated from *B. ferruginea* stem bark were found to display radical scavenging and xanthine oxidase inhibition activities, therefore supporting the application of *B. ferruginea* in traditional medicine [6] as an anthelmintic, antiamebic, antianaemic, antibacterial, anticonvulsant, antidiabetic, antidiarrhoeal, anti-inflammatory [21], antimicrobial [22], antiviral, hypoglycemic and for abdominal pain, cardiovascular, gynecological and sexual diseases [23] or anti-cancer [24]. Other reported activities of the bark extract include trypanocidal, molluscidal [25].

Honey is a natural sweet viscous fluid produced by honeybees from the pollen and nectar of flowering plants or from the nectar of blossoms which honeybees collect and transform by combining with their salivary secretions and deposit, dehydrate and store in the honey comb to ripen [26]. Some honey substances are essential for human life such as sugars (the major sugar present in all the types of honey is fructose), proteins, vitamins, organic acids and minerals [27]. Honey has been reported to have antibacterial activity against diarrhoeagenic bacteria [28, 29] and immunostimulatory potential [30].

According to Alfa *et al.* [31] traditionally used medicinal plants of ethno-pharmacological relevance can be a substantial source of drugs and thus are worthy of investigation for potential biomedicine development. Thus, this study is aimed towards better understanding of the pharmacotherapy property of formulated herbal mixture from *Sorghum bicolor* Linn., *Curcuma longa* Linn., *Bridelia ferruginea* Benth and honey.

MATERIALS AND METHODS

Sample Collection

The plants materials (*Sorghum bicolor*, *Curcuma longa* and *Bridelia ferruginea*) were bought from Oja-Oba in Ado Ekiti, Nigeria while honey was purchased from Pelumi Pure Natural Honey Farm in Ifaki Ekiti, Nigeria.

Plant identification was done at the herbarium at the School of Agriculture, Federal University of Technology, Akure, Nigeria.

Preparation of the Herbal Product

The plant materials were rinsed under running water and air dried at room temperature ($28\pm 2^{\circ}\text{C}$) to a constant weight before pulverizing into powder with electric blender (Hex Mokappi). Mixing ratio was determined according to Akande *et al.* [32], Haddad *et al.* [33] and Olarewaju *et al.* [34] as follows: *Sorghum bicolor*, (59.2%), *Curcuma longa* (18.5%), honey (19.2%), *Bridelia ferruginea* (3.1%).

Crude Extraction

Each powdered plant materials and honey were separately extracted with hot water and cold 70% ethanol. The filtrates were evaporated to dryness at 45°C using evaporating dish in a water bath. The crude extracts were then scrapped and stored in a sterile container in refrigerator [35].

Determination of Total Phenols

Total Phenolic compound was determined according to Tayyebe *et al.* [36] with a slight modification. An aliquot of 500 μL of the extract was transferred to a test tube, 500 μL of the Folin-Ciocalteu solution was added, then 1 mL of sodium carbonate solution and 8 ml of distilled water were also added. The samples remain at $28\pm 2^{\circ}\text{C}$ for 30 mins. Absorbance was measured at 760nm spectrophotometrically. These assays were performed in duplicates and the total phenolic content was expressed as milligrams of tannic acid equivalents per gram of sample (mg TAE/g).

Determination of Tannins

Casein (500 mg) was weighed and transferred into a 25 ml Erlenmeyer flask, 5 ml of the extract and 5 ml of distilled water were added. After 2h, the extracts were filtered into a 10 mL volumetric flasks and its volume was adjusted with distilled water. Then 500 μL of the extract was transferred to a test tube, 500 μL of the Folin-Ciocalteu solution was added, then 1 mL of sodium carbonate solution and 8 ml of distilled water were also added. The samples remain at room temperature for 30 mins. Absorbance was measured at 760nm spectrophotometrically. These assays were performed in duplicates and total tannins content is expressed as milligrams of tannic acid equivalents per gram of sample. (mg TAE/g) [36].

Determination of Flavonoids

An aliquot of 500 μ L of the extract was transferred to a test tube, 500 μ L of acetic acid solution, 2 mL of pyridine solution, 1 ml of aluminium chloride solution and 6 ml of 80% methanol were also added. The samples remain at $28\pm 2^{\circ}\text{C}$ for 30 mins. Absorbance was measured at 420nm spectrophotometrically. The test was carried out in duplicates and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of sample (mgQE/g) on dry weight basis using standard curve [37].

Determination of Saponins

Two grammes (2g) of the extract was weighed into a conical flask and 20ml of 20% aqueous ethanol was added. It was then heated over the water bath for 4 h with continuous stirring at 55°C . This mixture was then filtered and the residue was re-extracted with another 200ml of 20% ethanol. This combined extracts were reduced to 40ml over water bath at about 90°C . These concentrates were transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in the water bath. After evaporation the samples were dried in the oven to a constant weight to give the saponins which was then calculated according to Obadoni and Ochuko [38].

Determination of Alkaloids

Five grammes (5g) of the sample was weighed into 250 ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was then filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate was washed with dilute ammonium hydroxide and filtered. The residue which is the alkaloids was dried and weighed [39].

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capacity of the sample extract and standards were determined using the method of Boulanouar *et al.* [27]. At different concentrations (50–250 $\mu\text{g}/\text{mL}$), 2.5 mL of samples or standard were mixed with PBS (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1.0%). The obtained mixture was incubated at 50°C during 20 mins and TCA (2.5 mL, 10%) were added to the mixture. Afterward, 2.5 mL of this solution was mixed with distilled water (2.5 mL) and FeCl_3 (0.5 mL, 0.1%). The ferric/ferrous transformation was investigated, and the absorbance values were measured at 700 nm in a spectrophotometer.

GC-MS Analysis of FHP

A GC 6890N instrument from Agilent (Palo Alto, CA, USA) coupled with a mass detector (MS5973; Agilent) was used for the analysis of the sample. Experimental conditions of the GC-MS system were as follows: a DB 5MS column (30 m × 0.25 mm, 0.25 μm film thickness) was used and the flow rate of the mobile phase (He) was set at 1 mL/min. In the GC part, temperature was kept at 35°C for 8 min and then increased to 60°C at 6°C/min intervals followed by 4°C/min to 160°C and 20°C/min to 200°C/min and was kept at 200°C for 1 min. Organic compounds in the samples were identified in Wiley's NIST Mass Spectral Library if the obtained comparison scores were higher than 95%. Otherwise, fragmentation peaks of the compounds were evaluated, and the compounds were identified using the memory background for the identification of the compounds that appeared in GC-MS chromatograms. Contents of individual compounds in the sample were given in percent of the total compound in the sample. This was the standard way used to quantify the organic compounds present in FHP.

Determination of Drug probative and Drug Likeness

The drug likeness and properties of the suspected bioactive compounds of FHP was evaluated using online program Osiris Server [40].

Results

The present investigation revealed the presence of medicinally bioactive compounds in FHP. The phytochemical characteristics of FHP and the individual components of the product are presented in Tables 1 and 2. Alkaloids, phenols, saponins, tannins and flavonoids were found in the FHP and the different components used for the preparation of FHP irrespective of the extractant used. The highest values of alkaloids (9.57±0.01mg/g) and saponins (7.35±0.01 mg/g) were observed in the aqueous extract of FHP while the highest values of phenol (0.98±0.01 mg/g), tannins (0.68±0.00 mg/g) and flavonoids (40.54±0.05mg/g) were observed in the aqueous extract of turmeric.

The antioxidant activities of the materials investigated revealed that *B. ferruginea* (1956.05±15.54mgAAE/g) has the highest Ferric Reducing Antioxidant capacity followed by *S. bicolor* (1359.89±27.2 mgAAE/g) while FHP had the lowest (Table 3).

The results of the GC-MS and HPLC spectra of the aqueous extraction of FHP are shown in Figures 1 and 2 respectively while Tables 4 and 5 on the other hand show the drug properties and some selected bioactive compounds in the GC-MS and HPLC screening of FHP showing the presence of Gallic acid, Catechin, P-coumaric acid, Ferrulic acid, Rutin, Apigenin, Quercetin, Kampferol.

Discussion

The presence of phytochemicals in the individual components used for the preparation of FHP in this study agrees with the results of previous studies in which a myriad of phytochemicals were observed in *Sorghum bicolor* [32, 11], Turmeric [41, 42, 3], *B. ferruginea* [23, 35, 20, 6] and honey [43].

The phytochemical profiles of the aqueous extract of FHP as compared to the ethanol extracted FHP showed higher values of the all the phytochemicals investigated. This shows that aqueous extraction using hot water facilitated the release of the phytochemicals in FHP better than the use of ethanol and also in the release of the phytochemicals in the different components used for its preparation showing that hot water is a better extractant. This is in however in contrast with the report of Arawande *et al.* [42] who observed highest extractive value using ethanol and lowest using water. The differences in extractive value may be associated with the fact that the present study makes use of hot water. According to Chaves *et al.* [44], increase of water temperature produce dramatic changes in its physical-chemical properties thus enhancing the solubility of the analytes, breaking matrix-analyte interactions achieving a higher diffusion rate. Tena [45] also stated that with moderate elevated temperature, the polarity of water can be reduced considerably and can act as if ethanol or acetone were being employed. Specifically, the higher extractive value of water as compared to ethanol was observed in the yield of flavonoids and alkaloids in FHP and turmeric in this study. The highest phenolic content in turmeric however disagrees with the report of Mahomoodally *et al.* [6] who reported highest phenolic content in stem bark of *B. ferruginea*.

According to Ogonnia *et al.* [20], the antioxidant power of plant material is associated with the extractive value of flavonoids and is employed to manage various disease states with different oxidative stress aetiologies. The antioxidant activities as shown in Table 3 revealed that *B. ferruginea* ($1956.05 \pm 15.54 \text{ mgAAE/g}$) has the highest Ferric Reducing Antioxidant capacity followed by *S. bicolor* ($1359.89 \pm 27.2 \text{ mgAAE/g}$). This agrees with the previous study [46] while the observation that *S. bicolor* also has very high FRAC also corroborates the report of Kathleen *et al.* (2013) who stated that the antioxidant protection capacity of the leaf sheaths from *S. bicolor* is many-fold higher than that reported for cereal grains and vegetables

The presence of Gallic acid, Catechin, P-coumaric acid, Ferrulic acid, Rutin, Apigenin, Quercetin, Kampferol in FHP show that the herbal drug contains potent organic compounds that have therapeutic potentials. For instance, Gallic acid and Catechin been reported to have great potential to facilitate natural defense against microbial infections and modulate the immune response [47] while the potential of apigenin in West African *Sorghum bicolor* Leaf Sheaths in reducing inflammation-induced immune suppression has also been reported [11]. Catechin has also been shown to effectively inhibit influenza A (H1N1) virus infection and also has anti-hepatitis B virus activity [48]. Furthermore, rutin one of the compounds identified in this present study has also been reported to have strong inhibitory effect on the SARS-CoV-2 main protease (M^{pro}) [49] while ferrulic acid and quercetin have been reported to have a strong ant-viral potential [50, 49].

Table 1: Quantitative phytochemical profile of ethanolic extract of FHP and individual components

Sample Codes	ALKALOIDS (mg/g)	PHENOLS (mg/g)	SAPONINS (mg/g)	TANNINS (mg/g)	FLAVONOIDS (mg/g)
A (Bridiela)	5.09±0.03	0.79±0.01	2.44±0.11	0.30±0.02	7.57±0.05
B(Sorghum)	0.35±0.01	0.74±0.01	0.58± 0.04	0.63±0.02	26.56±0.27
C (Turmeric)	0.32±0.01	0.76±0.01	0.04±0.02	0.63±0.03	33.15±0.02
D(Honey)	0.596±0.02	0.26±0.02	5.94±0.02	0.26±0.03	0.34±0.02
FHP	0.32±0.01	0.26±0.03	0.02±0.004	0.14±0.04	3.17±0.45

Table presents mean and mean deviation

Table 2: Quantitative phytochemical profile of aqueous extract of FHP and individual components

Sample Codes	Alkaloid (mg/L)	Phenols (mg/L)	Saponins (mg/L)	Tannins (mg/L)	Flavonoids (mg/L)
A (Bridiela)	5.26±0.00	0.80±0.00	1.95±0.00	0.32±0.00	7.92±0.00
B (Sorghum)	0.48±0.00	0.86±0.00	0.90±0.02	0.53±0.00	28.16±0.04
C (Turmeric)	0.43±0.00	0.98±0.01	0.22±0.00	0.68±0.00	40.54±0.05
D (Honey)	0.485±0.01	0.33±0.03	0.11±0.01	0.18±0.01	2.935±10.2
FHP	9.57±0.01	0.59±0.01	7.35±0.01	0.21±0.01	23.78±0.08

Table presents mean and mean deviation

Table 3: Ferric Reducing Antioxidant Power (FRAP) of FHP and individual components

Sample	FRAP (mgAAE/g)
A (Bridiela)	1956.05±15.54
B (Sorghum)	1359.89±27.20
C (Turmeric)	485.03±13.41
FHP	359.89±3.88

Key: AAE means Ascorbic acid equivalent

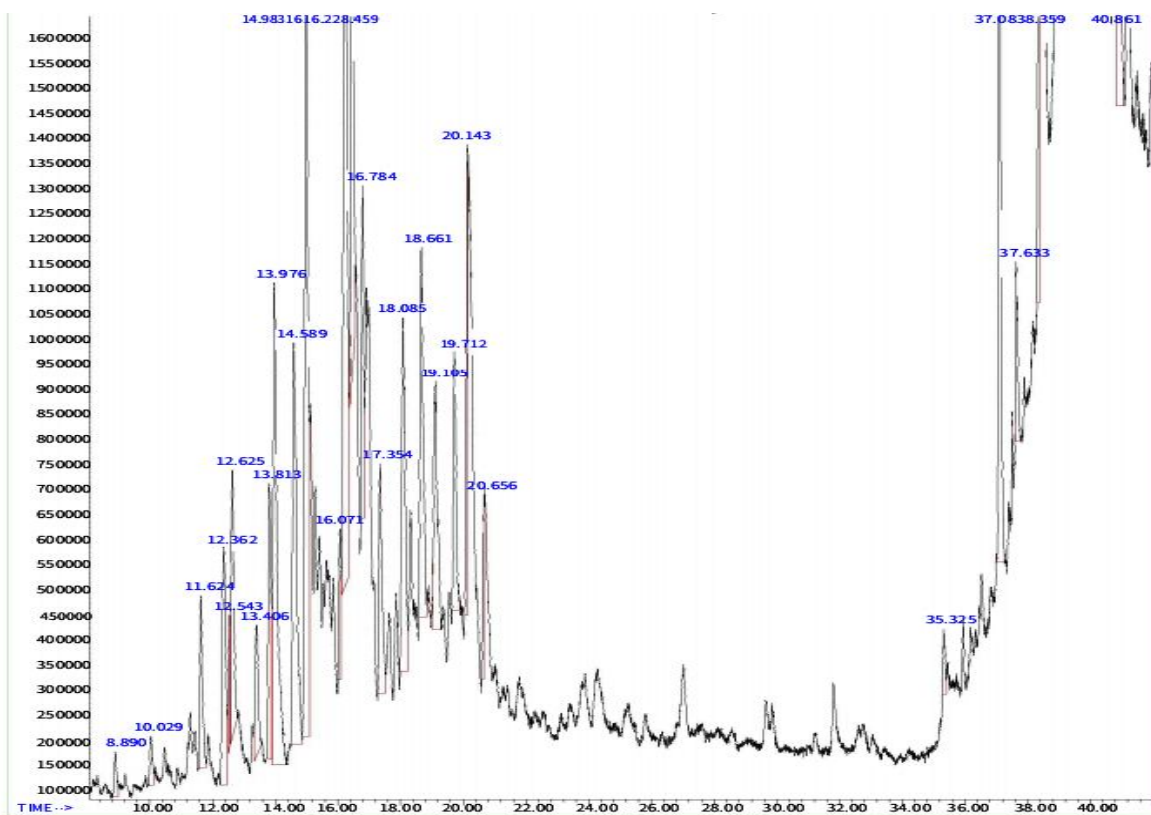


Fig. 1: Gas Chromatograph of FHP

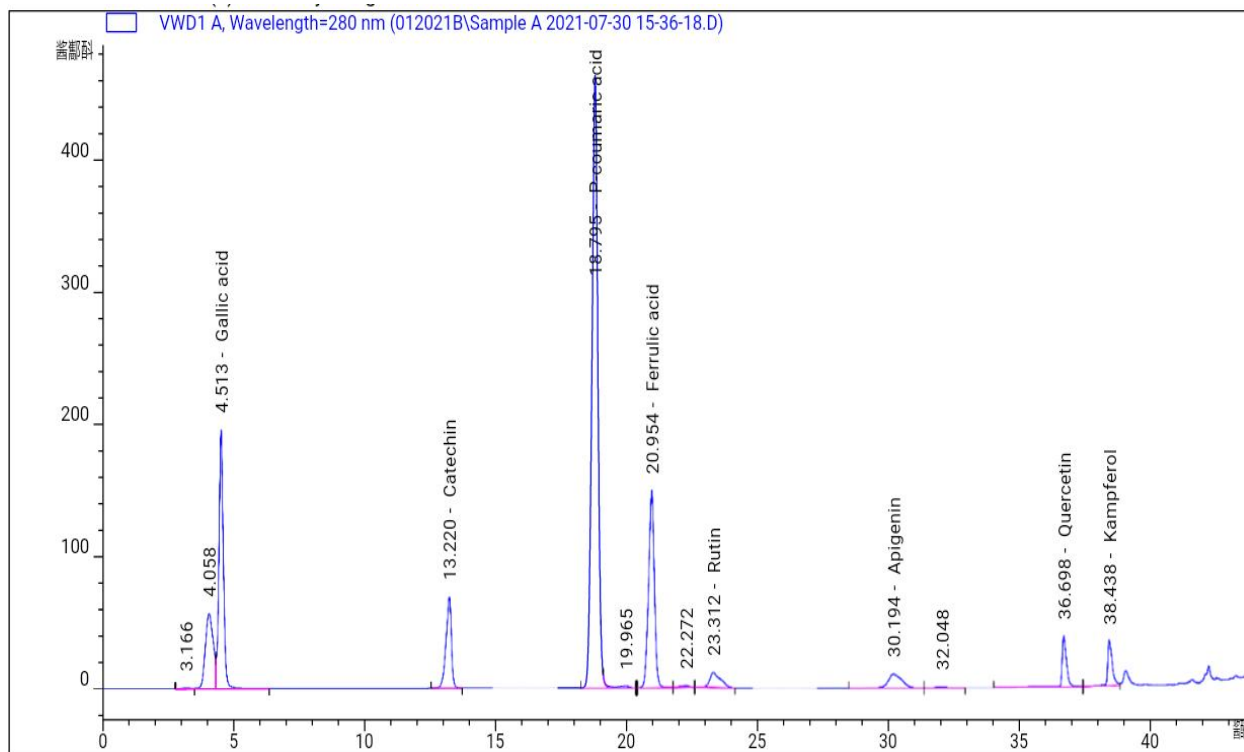


Figure 2: High Performance Liquid Chromatograph of flavonoid compound of FHP

Table 4: Drug properties of the identified compounds from GC-MS of FHP

S/N	Compound name	Drug likeness	Mutagenic	Tumorigenic	Irritability	Reproductive effect	Drug Score
1	O-Cymene	-2.5088	None	none	none	None	0.47
2	Sulfone	-0.165	None	none	none	None	0.50
3	1H-Indene	3.9929	None	none	none	None	0.49
4	Benzene, 1-ethyl-3-methyl-	-2.8	None	None	None	None	0.48
5	Benzene, 1-ethenyl-4-ethyl-	-4.99	None	None	Medium	None	0.35
6	Benzene, (2-methyl-1-propenyl)-	-3.18	None	None	None	None	0.45
7	Indan, 1-methyl-	-1.82	None	None	None	None	0.50
8	(E)-1-Phenyl-1-butene	-6.1	None	None	None	None	0.44
9	2-Ethyl-2,3-dihydro-1H-indene	-0.79	None	None	None	None	0.57
10	Bicyclo[4.2.1]nona-2,4,7-triene	-0.84	None	None	None	None	0.62

Table 5: Drug properties of the identified compounds in HPLC of the Flavonoid of FHP

Compound name	Drug likeness	Mutagenic	Tumorigenic	Irritability	Reproductive effect	Drug Score
Gallic acid	0.12	High	None	None	High	0.27
Catechin	1.92	None	None	None	None	0.87
P-coumaric acid	0.58	None	None	None	High	0.47
Ferrulic acid	1.12	High	High	None	High	0.18
Rutin	3.31	None	None	None	None	0.57
Apigenin	1.21	High	None	None	None	0.47
Quercetin	1.6	High	High	None	None	0.3
Kampferol	0.9	High	None	None	None	0.46

CONCLUSION

This study has shown that the formulated herbal product prepared by our team has bioactive constituents such as Catechin, P-coumaric acid, Ferrulic acid, Rutin, Apigenin, Quercetin and Gallic acid. The implication of this is that the formulated herbal product has good medicinal potentials.

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