

# Preliminary Phytochemical Studies, GC-MS Analysis and *In vitro* Antioxidant Activity of Selected Medicinal Plants and its Polyherbal Formulation

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## ABSTRACT

**Background:** Novel polyherbal formulation (PHF) is the utilization of more than one herb in the preparation of herbal medication. The thought is found in the conventional system of medicine where the variety of herbs in a specific proportion of illness. Because of synergism, polyherbalism presents a few advantages which aren't accessible in single herbal medication. It is utilized in these medications for the treatment of numerous sicknesses including antioxidants. **Objective:** To develop a phytochemical screening and GC-MS analysis of Novel Polyherbal formulation for *In vitro* antioxidant activity. **Materials and Methods:** Macroscopical, preliminary phytochemical, quantitative phytoconstituents, and *In-vitro* antioxidant activity of all the individual extract and polyherbal formulation was done by chemical method. Identification of phytoconstituents with the aid of Gas chromatography – Mass spectroscopy (GC-MS). **Results:** Macroscopical study and physicochemical examination, for example, ash value, extractive value, loss on drying, and pH were reported to *A. racemosus*, *B. variegata*, *C. bonducella*, *S. asoka*, and *S. racemosus* and novel polyherbal formulation. Qualitative phytochemical investigation revealed the presence of alkaloids, flavonoids, gums & mucilage, carbohydrates, steroids, proteins & amino acids, fats & fixed oils, glycoside, phenols, and saponins. Quantitative estimation such as TAC, TFC, TGC, TSC, and TPC was showed positive results. All the individual extract and PHF were subjected to GC-MS analysis. All the individual extract and polyherbal formulation displayed strong antioxidant activity. **Conclusions:** To conclude the PHF was reported that high level of bioactive contents present and strong antioxidant activity in contrast to the preferred ascorbic acid. The GC-MS uncovered the presence of bioactive compounds and these compounds are suggested to treat antibacterial, antioxidant, anti-inflammatory, and antiviral, anti-tumor, anti-proliferative activity, and antifungal activity.

**Key words:** Phytochemical, Macroscopical, Antioxidant, Polyherbal formulation, GC-MS analysis.

## INTRODUCTION

The conventional medication everywhere in the world is nowadays uncovered by an in-depth movement of researchers on various plant species and their restorative principles. The conventional medication everywhere in the world is these days revealed by a substantial action researcher on various plant species and their therapeutic principles. Plants contain phytochemicals with various bioactivities including antioxidant, anti-inflammatory, and anticancer activities. Right now, about 25% of the active component was recognized from plants that are utilized as prescribed medicines.<sup>1</sup> Oxidative stress is a critical danger factor in the pathogenesis of various chronic diseases. An antioxidant can be extensively characterized as the substance that delays or impedes oxidative harm to an objective particle. The most characteristic of an antioxidant is its capacity to trap free radicals.<sup>2</sup> Natural antioxidants can shield the physical body from free radicals and retard the advancement of numerous long-term diseases. Because of the impact on the immune

structure of the human body, there is a requirement for natural antioxidants agents when contrast to artificial antioxidants (harmful for people). Plants contain numerous constituents with a nearby actual effect on body tissues, and therefore the topical use of herbal remedies is among the foremost noticeable within the simplest conventional health care system. To help the usage of selected plant extracts within the conventional system of medicine, the antioxidant capability of the rhizome of *Asparagus racemosus*, the bark of *Bauhinia variegata*, seed kernel of *Caesalpinia bonducella*, the bark of *Saraca asoka*, and hardwood of *Symplocococcus racemosus* was examined.<sup>3</sup>

In the current study, five different herbal plants were chosen for the preparation of polyherbal formulation and standardization in terms of macroscopical, physicochemical, qualitative, and quantification of bioactive constituents. All the individual plant extract and the polyherbal formulation were subjected to GC-MS analysis to consider the phytocompounds present and the evaluation of antioxidant activity was studied by the DPPH, radical scavenging, and hydrogen peroxide assay.

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*Asparagus racemosus* (family Asparagaceae) commonly referred to as Shatavari, Satawar, Thaneervitan kilangu, Satamuli, is an indigenous herb found in India. Shatavari is a tremendous herb in Ayurveda, is understood as the Queen of herbs since it promotes love and affection. Medicinal uses of Shatavari are mentioned referred to as nervous disorders, diuretic, stomachic, dyspepsia, diarrhea, dysentery, tumors, carminative, inflammation, galactagogue, hyperdipsia, stomachic, neuropathy, antiseptic, hepatopathy, cough, bronchitis, hyperacidity, and tonic. Reported Pharmacological activities of Shatavari contain antiulcer, antitussive, adaptogenic, antioxidant, antidiabetic, antiulcer, antiarrhoeal, antiprotozoal, antihepatotoxic, antineoplastic, cardiovascular effects, a versatile female tonic, and immunomodulatory activities.<sup>4</sup>

*Bauhinia variegata* (family Leguminosae) is usually referred to as the Orchid tree, Mandarai, Mountain ebony, and camel's food. It had been in their showy flowers and decorative foliage. It had been native to the Southeast and grows during a tropical and subtropical climate. Whole plant parts having medicinal value. Traditional uses of mountain ebony were astringent, tonic, bronchitis, leprosy, tumor, anthelmintic, diarrhea, piles, and antidiabetic. Pharmacological activities of *Bauhinia variegata* showed that antioxidant, anticancer, hypolipidemic, anti-inflammatory, antiulcer, antimicrobial, antiulcer, hepatoprotective, molluscicidal, nephroprotective, immunomodulating, and wound healing effects.<sup>5</sup>

*Caesalpinia bonducella* (family Caesalpiniaceae) may be a thorny bush broadly dispersed everywhere within the world uniquely, in India, Srilanka, Andaman, and therefore the Nicobar Islands, in India especially originate in a humid region. All parts of the plant have valuable medicinal plant; it had been utilized in traditional system of medicine. Reported medicinal properties of *Caesalpinia bonducella* was anti-inflammatory, antitumor, antimalarial, antifungal, antidiabetic, antispasmodic, antioxidant, antiproliferative, larvicidal, muscle contractile, anticonvulsants, adaptogenic, anxiolytic, antipsoriatic, antifilarial, and antispasmodic.<sup>6</sup>

*Saraca asoka* (family Caesalpiniaceae) generally called Ashoka may be a Sanskrit word that meaning "without sorrow" or which that provides no grief. It's also referred to as Asogam (Tamil), Asokam (Malayalam), Asokapatta (Telugu), and Ashok (Kashmiri). Reported pharmacological activities of Ashoka included anti oxytoxic, anti-microbial activity, anti-menorrhagic, and anti-cancer.<sup>7</sup>

*Symplocos racemosa* (family Symplocaceae) is usually referred to as Lodhra in Sanskrit. Traditional use of *Symplocos racemosa* was stomachic, expectorant, astringent, anti-inflammatory, febrifuge, hemostatic, and constipating. It's useful in leprosy, disease of the skin, tumors, asthma, bronchitis, arthritis, fever, pimples, hemorrhages, diarrhea, baldness, ear diseases, elephantiasis, and gonorrhoea.<sup>8</sup>

## MATERIALS AND METHODS

### Collection and authentication of plants

All the ingredients of the Polyherbal formulation are collect and purchased from different parts of Chennai. Their authentications were confirmed in the Botanical Survey of India, Coimbatore by comparing their morphological and microscopical characters with those given in the ancient literature and books.

### Macroscopic analysis

Organoleptic and macroscopic analysis of *A. racemosus*, *B. variegata*, *C. bonducella*, *S. asoka*, and *S. racemosa* were performed and the parameters evaluated for the different parts of color, odor, taste, shape, and texture were observed and noted.<sup>9,10</sup>

### Method of preparation polyherbal formulation

All the selected medicinal plant parts were cleaned by utilizing a sterilized fabric cloth to get rid of dirt and via air, blustering to eliminate minute sand particles. Each 1000 mg of a polyherbal formulation contains a different quantity of *Asparagus racemosus* (root), *Bauhinia variegata* (wood), *Caesalpinia bonducella* (seed kernel), *Saraca asoka* (Bark), and *Symplocos racemosus* (wood). Each plant material was size reduced using the blender. After that sieved in separately all the plant materials it was using sieve no 60. Each blended and dried powder of individual plant materials was weighed in the required quantity. After that, all the individual powder was mixed in a geometrical type of mixing. 10 g of that polyherbal mixture was macerated with hydroalcoholic solvent (30:70) with infrequent stirring for 72 hrs. After 72 hrs the suspensions were shifted through a fine muslin fabric cloth and the collected filtrate was evaporated to dryness kept at desiccator. The yield of the collected polyherbal formulation was found to be 15.47% and was stored in an air-tight container for further analysis.<sup>11</sup>

### Physicochemical parameters

Physicochemical Constants of the Individual drugs and Polyherbal formulations have been done to estimate the quality and purity of the powder drugs. Physicochemical constants include Ash value it represents the occurrence of inorganic salts existing in the plant material. The extractive values included such as water-soluble and alcohol soluble extractive values were determined. Loss on drying and pH was carried out. The information composed since this evaluation was helpful for standardization and obtaining the quality standards for crude drugs as well as for polyherbal formulation. Determinations of these physicochemical constants were done according to the methodology referenced by WHO guidelines.<sup>11,12</sup>

### Preliminary phytochemical screening

All the individual plant extracts and Polyherbal mixer were subjected to screen the preliminary phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins, terpenoids, steroids, tannins, fatty acids, protein, and carbohydrate according to the standard methods.<sup>10</sup>

### Quantitative phytochemical analysis

A systematic and complete investigation of crude drugs should contain a detailed study of both primary and secondary metabolites derivative as an outcome of plant metabolism. All the individual extract and polyherbal were subjected to qualitative and quantitative phytochemical analysis such as alkaloid, flavonoid, steroid, saponin, phenolics, gums & mucilage, fats & fixed oils, carbohydrates, proteins & amino acids, and volatile oils were done using the prescribed method.

### Determination of total flavonoid content (TFC)

Determination of total flavonoid content depended on the aluminium chloride ( $\text{AlCl}_3$ ) method.<sup>13</sup> Taken a 50 mg standard quercetin component and dissolved in 50 ml methanol solution and different aliquots of 5-25 $\mu\text{g}/\text{ml}$  were prepared in methanol. It was utilized as a standard solution. 10 mg of dried individual plant extract and polyherbal formulation were dissolved in 10 ml of methanol and filter. 3 ml of (1mg/ml) of this extract was utilized for the estimation of flavonoids. In the last advance, take 3 ml of plant extract or standard and add 1 ml of 2%  $\text{AlCl}_3$  methanolic solution. This combination of the mixture is allowed to stand for 60 min at room temperature. Then absorbance was measured at 420 nm by utilizing a spectrophotometer.

### Determination of total alkaloid content (TAC)

The individual plant extract and polyherbal formulation (1mg/ml) were thawed in 2 N HCL and afterward shifted to a separating tube. The pH of the phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 ml of sample solution was lifted to a separating funnel and afterward, 5 ml of BCG solution together with 5 ml of phosphate buffer were added. The combined blend was shaken were gathered in a 10 ml volumetric jar and weakened to volume with chloroform. The absorbance of the complex in chloroform was estimated at 470 nm.<sup>14</sup>

### Determination of total steroidal content (TSC)

The determination of steroidal substance was done by Liebermann-Burchard colorimetric assay method with minor modifications utilizing as a standard.<sup>15</sup> The Liebermann Burchard reagent was set up by adding 5 ml of concentrated sulphuric corrosive to 50 mL of acidic anhydride solution. All the extracts and polyherbal formulation were diluted with chloroform and to the chloroform extract, freshly prepared Liebermann-Burchard reagent was added and estimated at 650 nm against a reagent blank. Steroidal content was expressed in mg of cholesterol equal to g of dry load of the extract.

### Determination of total glycosidal content (TGC)

The determination of glycoside content was completed utilizing a Baljet reagent indicated by the method described in Nandhini *et al* 2020 1 mL of extract was added to the solution of Baljet reagent containing picric acid and 0.1 N sodium hydroxide with the proportion of 95:5. The solution was permitted to incubate in a dark chamber for 60 min and additionally diluted to 15 ml with distilled water and absorbed at 495 nm. Digitoxin was utilized as a standard for assurance of glycoside and the results were communicated in mg of digitoxin equal to g of the dry weight of the extract.<sup>16,17</sup>

### Determination of total saponin content (TSC)

The determination of saponin content was based on Nandhini *et al* 2020 utilizing diosgenin as a standard solution.<sup>17</sup> 1 mL of 80% aqueous methanol was added to 1 mL of diluted extracts and polyherbal formulation followed by 1 mL of 72% sulphuric acid was added to the sides of the test tubes. The blend was warmed on a water bath 60°C for 10 min and the absorbance was recorded at 544 nm against 80% methanol as a blank solution. The total saponin content was determined utilizing a standard calibration curve of diosgenin with a concentration range of 20-200 µg/mL solution and the outcomes were expressed in mg of diosgenin equivalent to g of the dry weight of the extract.

### Determination of total phenolic content (TPC)

The microplate total phenolic content method was determined by the 96 – well microplate Folin-Ciocalteu procedure adjusted from Sembiring *et al* with specific alterations.<sup>18</sup> A whole of 25 µL of the individual extract and polyherbal formulation (diluted form) were blended in with 100 µL of 1:4 weakened Folin-Ciocalteu reagent and shaken for 60 sec in a level base 96-well microplate. The collective blend was left for 4 mins and afterward, 75 µL of sodium carbonate solution (100g/L) was added and the combination was shaken at a medium constant speed for 1 min. After 2 h at room temperature, the absorbance was assessed at 765 nm utilizing the microplate reader. The absorbance of a similar response with ethanol rather than the sample and standard was deducted from the absorbance of calibration. Complete phenolic contents were stated as mg Gallic Acid Equivalents (GAE) per g of extracted plant samples and polyherbal formulation.

### GC-MS analysis

For the identification of the phytochemical compounds, the hydroalcoholic extract of individual plants and PHF was exposed

to the examination of GC-MS analysis. Gas chromatography-Mass spectrometry (GC/MS) was carried out in the Shimadzu 17A GC combined with Shimadzu QP2010 plus (quadrupole) Mass Spectrometer (Shimadzu, Japan), furnished with EI and a fused silica column DB-5 (30m×0.25 mm i.d) of 0.25µm film thickness was required. The oven temperature at 500°C for 5 minutes and then modified from 50-2800°C for 40 minutes. High pure Helium was used as a carrier gas for this analysis. The flow rate of helium gas was used at 2 mL/min, with the split proportion of 1:30 mode was utilized for sample injection of 1µl and ionization voltage of MS-analysis was controlled by EI procedure at 70 eV. The Phytochemical constituents were recognized by associating the results of the mass spectrum with the inbuilt NIST library database.<sup>19-22</sup>

### *In vitro* antioxidant activity

#### DPPH method

All the individual plant extracts and PHF was subjected to the DPPH free radical scavenging assay was determined by the technique depicted by the method Madhu SE *et al*<sup>23</sup> slight adjustments. This DPPH assay quantifies the capacity of all the extract and polyherbal formulation below assessment to scavenge the free radicals. All the five extracts and polyherbal formulation were calculated for the antioxidant activity against DPPH free radical scavenging assay. The stock solution of all the extracts and standard solution (ascorbic acid) was prearranged for the concentration of 1 mg/ml. Three serial dilutions of each extract and polyherbal formulation and standard ascorbic acid were made (12.5µg/ml, 25µg/ml, 50µg/ml, 100 µg/ml, and 200 µg/ml). Every 3 ml of each extract and the standard solution were added to the 1 ml of DPPH solution (0.1Mm/L). This mixed solution was shaken forcefully and incubated for 20 min in a dark room. This incubated solution was estimated the absorbance was noted at 517 nm. The whole procedure was repeated three times. The IC<sub>50</sub> value of DPPH assay was calculated using the below formula:

$$\% \text{ Inhibition of DPPH assay} = (Ac - As / Ac) \times 100$$

Where Ac = Absorbance of Control

As = Absorbance of Standard / Sample

#### Hydrogen peroxide assay

The ability of all the individual extracts and the polyherbal formulation was estimated according to the method given by Ruch RJ *et al* and Saumya SM *et al.*<sup>24,25</sup> Using phosphate buffer H<sub>2</sub>O<sub>2</sub> solution was prepared and maintain pH at 7.4. All the individual extracts and PHF (12.5µg/ml, 25µg/ml, 50µg/ml, 100 µg/ml, and 200 µg/ml) were added 0.6 ml of H<sub>2</sub>O<sub>2</sub> solution. Ascorbic acid was used as the standard. The absorbance of H<sub>2</sub>O<sub>2</sub> at 230 nm was estimated after against without adding H<sub>2</sub>O<sub>2</sub> solution and associated with ascorbic acid was used as the reference compound.

$$H_2O_2 \text{ activity (\%)} = (Ac - As / Ac) \times 100$$

Where Ac = Absorbance of Control

As = Absorbance of Standard / Sample

#### Reducing power assay

Each extract and PHF was subjected to the reducing power assay based on the method derived from Gülçin İ *et al*, Meriga Bet *al.*<sup>26,27</sup> Each sample and standard ascorbic acid (12.5µg/ml, 25µg/ml, 50µg/ml, 100 µg/ml, and 200 µg/ml) was added 1ml of distilled water this mixture was sonicated at 20 mins for aqueous extraction. In this above solution mixed with 2.5 ml of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>]. The combined solution was incubated for 20 min at 50°C. 2.5 ml of above the upper layer solution was blended in with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance was estimated at 700 nm.

## RESULTS AND DISCUSSION

### Phytochemical analysis

#### Macroscopical evaluation

The macroscopical evaluation was carried out to assess the color, odor, taste, shape, and texture of the individual drugs, and the polyherbal formulation was observed and recorded in Table 1.

#### Physicochemical Analysis

Physicochemical analysis of individual ingredients and PHF was studied and represented with standard deviation. In physicochemical evaluation such as total ash, water-soluble ash, acid insoluble ash, water-soluble extractive value, ethanol-soluble extractive value, loss on drying, and pH were evaluated results were given in Table 2. The ash values demonstrate the presence of inorganic salts present in the drug. The extractive values (water and ethanol soluble extractive value) were resolved. The data gathered from this evaluation was helpful for standardization and obtaining the quality standards for a crude drug as well as for PHF formulations. Determination of these physicochemical constants was according to systems referred to as per WHO guidelines.

#### Preliminary phytochemical screening

Preliminary phytochemical screening of the individual drugs and polyherbal formulation confirmed the presence of phytoconstituents such as flavonoids, alkaloids, carbohydrates, gums & mucilage, fats & fixed oils, steroids, glycosides, phenols, saponins but no volatile oils (Table 3).

#### Determination of bioactive contents

The quantitative determination of bioactive contents includes alkaloid, flavonoid, glycoside, steroid, saponin, and phenol were determined in the hydroalcoholic extract of individual drugs, and PHF results were given in Figure 1 and described in Table 4.

Alkaloids were equivalent to Atropine, phenolics equivalent to Gallic acid, flavonoids equivalent to Catechin, glycosides equivalent to Digitoxin, steroids equivalent to Cholesterol, and saponins equivalent to Diosgenin.

#### GC-MS profile

The GC-MS analysis in the hydroalcoholic extract of *Asparagus racemosus* showed the presence of major phytochemical compounds

**Table 1: Organoleptic Description of Individual plant part in polyherbal formulation.**

Parameters	AR	BV	CB	SA	SR
Part to be used	Root	Bark	Seed	Bark	Wood
Color	Brown colored	Externally brownish and internally light reddish-brown.	Greenish gray to bluish-gray	Grayish brown	Grayish brown
Odor	Characteristic	Characteristic	Characteristic	Aromatic	Characteristic
Taste	Mucilaginous	Mucilaginous followed by bitter sensation.	Bitter	Astringent	Astringent
Shape	Tuberous & elongated	Curved	Globules or round	Vary in size	Slightly Curved
Texture	Soft & contains epidermal hairs	Hard surface	Smooth and shiny	Hard and strong	Smooth

AR-*Asparagus racemosus*, BV-*Bauhinia variegata*, CB-*Caesalpinia bonducella*, SA-*Saraca asoka*, SR-*Symplocococus racemosus*, PHF-Polyherbal formulation

**Table 2: Physicochemical constants.**

Parameters	AR	BV	CB	SA	SR
Total ash (%w/w)	3.00 ± 0.143	9.5 ± 1.34	3.52 ± 0.241	4.70 ± 0.183	10.03 ± 1.003
Water soluble ash (%w/w)	1.83 ± 1.271	3.48 ± 0.28	2.03 ± 1.002	2.62 ± 0.382	3.12 ± 2.041
Acid insoluble ash (%w/w)	0.29 ± 0.302	0.63 ± 0.07	0.58 ± 0.042	0.15 ± 0.041	0.37 ± 1.052
Water soluble Extractive value (%w/w)	12.92 ± 0.824	14.03 ± 1.002	16.54 ± 1.801	10.71 ± 1.031	9.17 ± 0.401
Ethanol soluble extractive value (%w/w)	7.82 ± 1.026	6.6 ± 0.062	7.53 ± 0.034	8.42 ± 0.721	6.87 ± 0.521
Loss on drying (%w/w)	5.12 ± 0.407	4.38 ± 1.034	5.03 ± 0.104	3.55 ± 0.242	3.82 ± 1.052
pH	5.03 ± 0.381	5.14 ± 0.241	4.13 ± 1.316	5.77 ± 0.469	4.89 ± 0.041

Values were in mean ± standard deviation, n=3

AR-*Asparagus racemosus*, BV-*Bauhinia variegata*, CB-*Caesalpinia bonducella*, SA-*Saraca asoka*, SR-*Symplocococus racemosus*, PHF-Polyherbal formulation

**Table 3: Preliminary phytochemical analysis.**

Test of constituents	AR	BV	CB	SA	SR	PHF
Flavonoids	-	+	+	+	+	++
Alkaloids	-	+	-	+	+	++
Carbohydrates	+	+	+	+	+	+
Gums & Mucilage	+	+	+	+	+	+
Proteins & Amino acids	+	+	+	+	+	+
Fats and fixed oils	-	-	+	-	-	+
Steroids	+	+	+	+	+	++
Glycosides	-	+	-	+	+	+
Phenols	+	-	+	+	-	+
Saponins	+	-	+	-	-	+
Volatile oils	-	-	-	-	-	-

(+)- Present; (++)- Strongly present; (-)- Absence

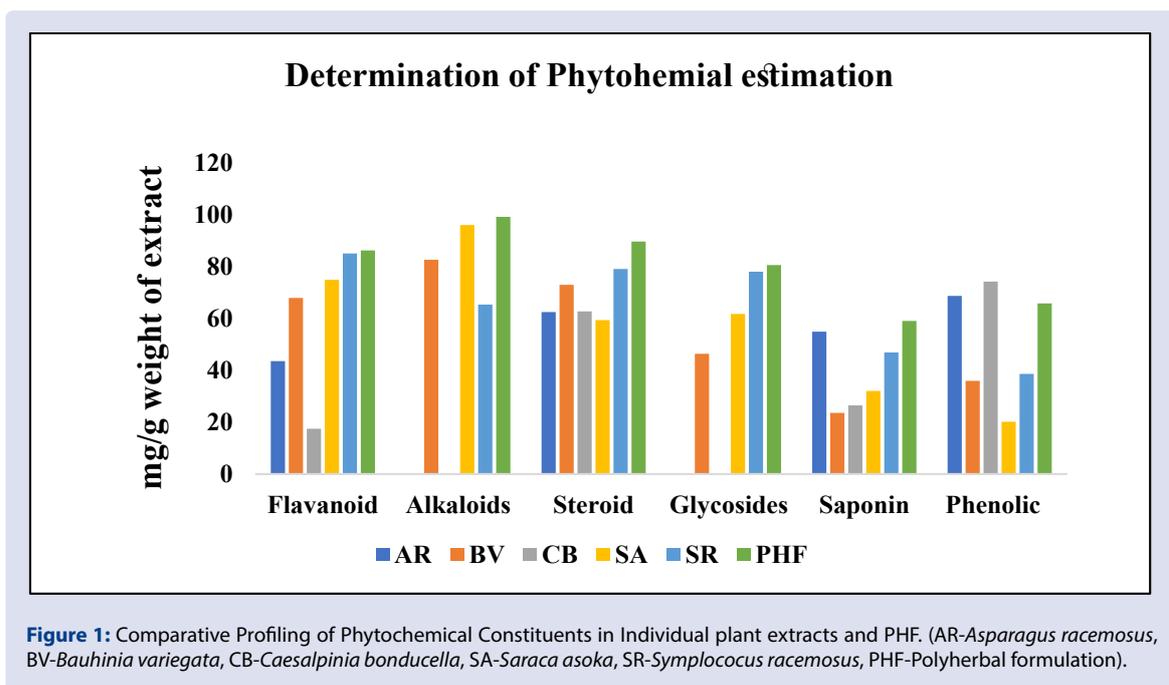
AR-*Asparagus racemosus*, BV-*Bauhinia variegata*, CB-*Caesalpinia bonducella*, SA-*Saraca asoka*, SR-*Symplocococus racemosus*, PHF-Polyherbal formulation

**Table 4: Determination of bioactive contents (mg/g).**

Quantitative parameters	Alkaloid	Flavonoid	Glycoside	Steroid	Saponin	Phenol
AR	ND	30.43 ± 0.97	ND	63.61 ± 1.17	55.6 ± 0.89	69.1 ± 0.42
BV	82.55 ± 0.78	68.36 ± 0.77	47.59 ± 1.12	72.03 ± 0.92	24.61 ± 0.93	36.53 ± 0.68
CB	ND	18.31 ± 0.78	ND	63.79 ± 0.97	26.9 ± 0.58	74.32 ± 0.54
SA	96.07 ± 0.64	74.1 ± 0.67	62.76 ± 1.01	51.42 ± 1.15	33.45 ± 1.13	20.59 ± 0.44
SR	66.49 ± 0.99	85.27 ± 0.57	79.261.36	80.53 ± 1.4	47.18 ± 0.62	38.71 ± 0.62
PHF	99.93 ± 0.78	87.26 ± 0.92	80.87 ± 0.36	90.52 ± 0.84	60.2 ± 0.98	66.62 ± 0.89

Values were in mean ± standard deviation, n=3

ND- Not Detected, AR-*Asparagus racemosus*, BV-*Bauhinia variegata*, CB-*Caesalpinia bonducella*, SA-*Saraca asoka*, SR-*Symplocococcus racemosus*, PHF-Polyherbal formulation

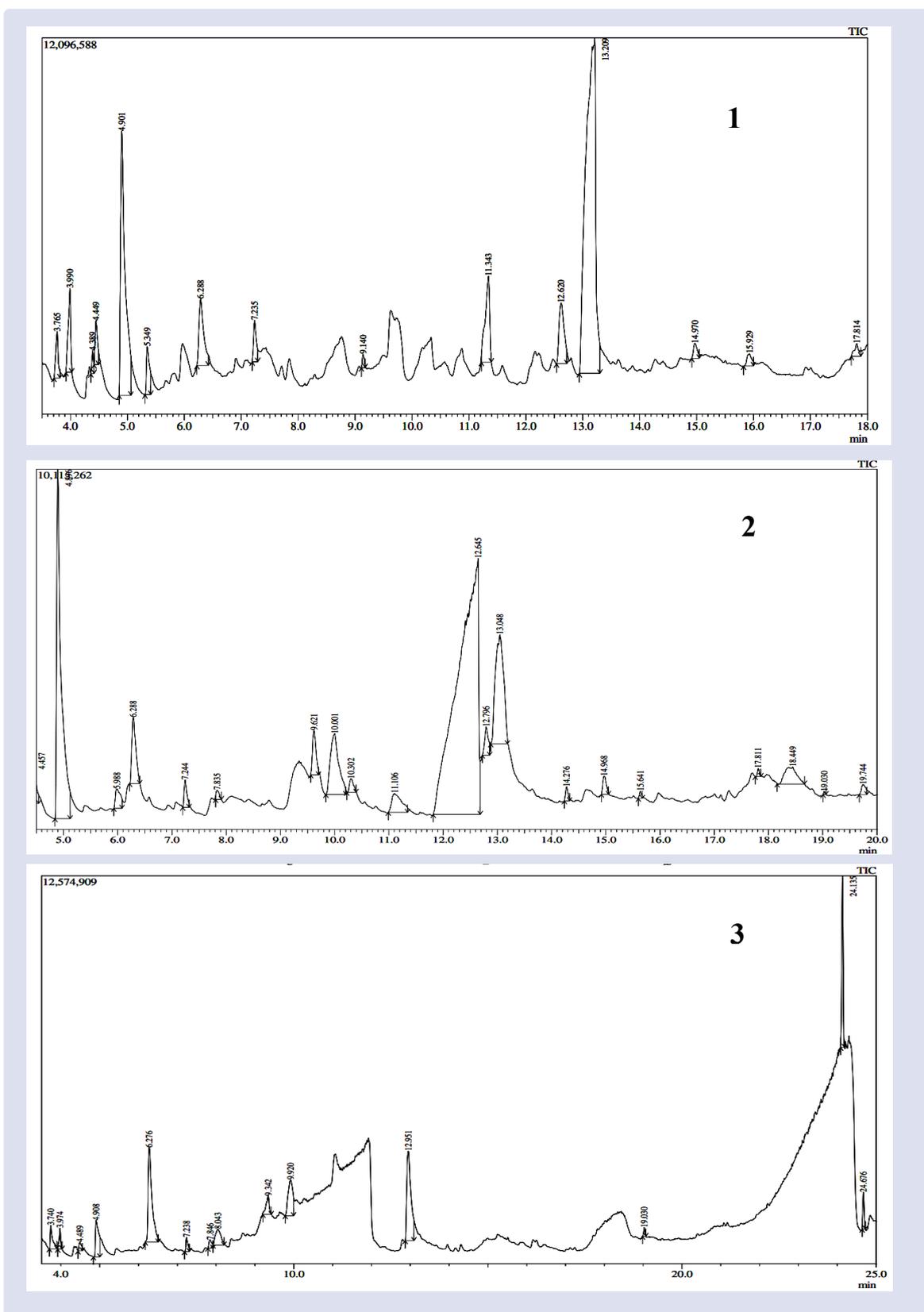


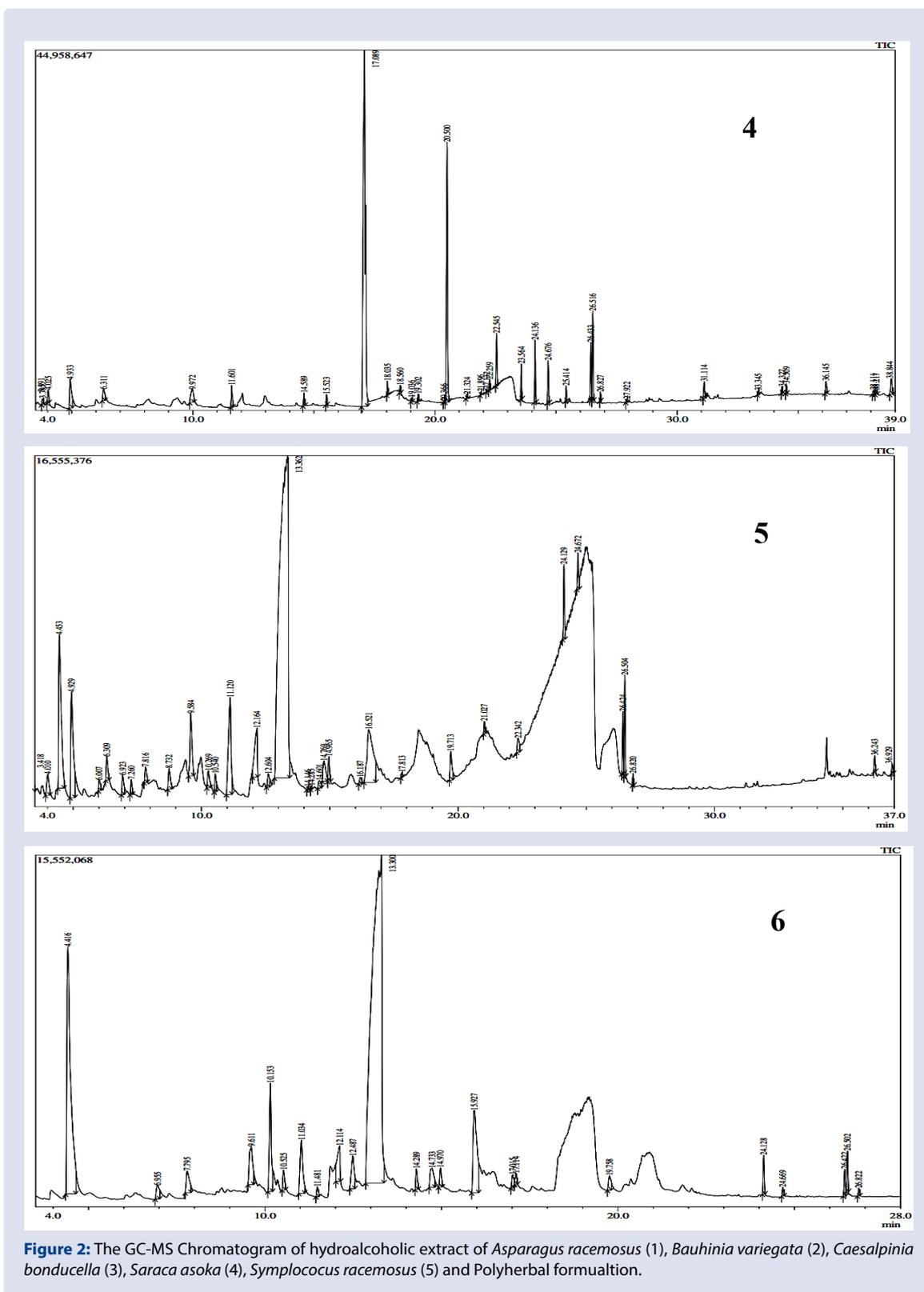
such as 2,2'-Bioxirane, 2-Furanmethanol, 6-Oxabicyclo (3.1.0) Hexan-3-one, 4-H-Pyran-4-one, 2,3 dihydro-3,5 dihydroxy-6, Isosorbide, 5-Hydroxy methyl furfural, D-Glucitol, and 1,4-anhydrous and 9,12-Octadecadienoic acid. *Bauhinia variegata* extract showed the presence of a variety of phytoconstituents such as 2-Furanmethanol, 2(5H)-Furanone 5-methyl-(Identit), Diazene, bis (1, 1-dimethyl ethyl), Benzoic acid, 5-Hydroxy methyl furfural, Hexadecanoic acid, methyl ester, and 9-Octadecenoic acid (Z) methyl ester. *Caesalpinia bonducella* extract exhibited the presence of 2(3H)-Furanone, 5-Methyl, 5-Hydroxy methyl furfural, 9, 12-Octadecadienoic acid methyl, 9, 12-Octadecadienoic acid (z,z)-2-hydroxy-1, Retinol acetate, and Rhodopin. Hydroalcoholic extract of *Saraca asoka* displayed the presence of Furfural, 2-Furanmethanol, Bicycle (2,2,1) Heptane-2-Carboxyl, Benzoic acid, 5-Hydroxymethyl furfural, and 1,2,3-Benzenetriol. *Symplococcus racemosus* showed the presence of 2-Furancarboxaldehyde, 3, 5-octadien-2-one, Levoglucosone, 4H-Pyran-4-one, 2,3-dihydro 3,5-dihydroxy-6, 5-Hydroxy methyl furfural, and Phenol 4-Propyl. The polyherbal formulation showed the presence of 2-Furanmethanol, 1-chlorodecane, Tetradecane 1-chloro, Methoxyacetic acid, pentadecyl ester, Hexadecanoic acid, methyl ester, 1, 2-benzene dicarboxylic acid, and 9,12-octadecadienoic acid. This analysis identified the pharmacological activity of reported and non-reported phytochemical compounds. Among the compounds, here mentioned only reported pharmacological activity of compounds. The active principle compounds, their retention time (RT), molecular

formula (MF), molecular weight (MW), peak area, and biological activity are presented in Figure 2 and Tabulated in Table 5-10.

### *In vitro* antioxidant activity

This DPPH assay is based on scavenging of the free radical from the antioxidants, which delivers a diminishing absorbance at 517 nm. All the individual extract and PHF displayed a comparable antioxidant activity with that of standard ascorbic acid at the different concentrations tested (12.5, 25, 50, 100, 200 µg/ml). Ascorbic acid was utilized as the standard drug for the estimation of antioxidant activity by the DPPH Scavenging method. The scavenging activity of DPPH free radical assay of standard ascorbic acid and hydroalcoholic extract of *A. racemosus*, *B. variegata*, *C. bonducella*, *S. asoka*, *S. racemosus*, and PHF were mentioned in Table 11 and Figure 3 (a). This free radical assay observed that all the individual extracts and PHF showed significant DPPH scavenging activity against free radicals. The H<sub>2</sub>O<sub>2</sub> scavenging assay was perceived and compared with the standard component of ascorbic acid. It is subsequently naturally favorable for cells to control the measure of hydrogen peroxide that is permitted to aggregate.<sup>28</sup> The antioxidant activity of the H<sub>2</sub>O<sub>2</sub> assay was depicted in Table 13 and Figure 3(b). Radical scavenging activity in a hydroalcoholic extract of *A. racemosus*, *B. variegata*, *C. bonducella*, *S. asoka*, *S. racemosus*, and PHF demonstrate the potential inhibitory effect of radical scavenging activity. The radical scavenging assay of all the individual extract and





**Table 5: Compounds identified by GC-MS in Hydroalcoholic extract of *Asparagus racemosus*.**

S.no	RT	Name	MF	M.W (g/mol)	Peak area %	Biological activity
1	3.321	2,2'-Bioxirane	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86.09	3.05	Antineoplastic
2	4.901	2-Furanmethanol	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98.1	10.06	Antiviral activity
3	6.288	6-Oxabicyclo (3.1.0) Hexan-3-one	C <sub>7</sub> H <sub>12</sub> O	98.1	2.57	Antibacterial
4	11.343	4-H-Pyran-4-one, 2,3 dihydro-3,5 dihydroxy-6	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144.12	3.15	Antioxidant, automatic nerve activity, anticancer, anti-inflammatory
5	12.620	Isosorbide	C <sub>6</sub> H <sub>10</sub> O <sub>4</sub>	146.14	2.55	Anti-inflammatory and cardiovascular disease
6	13.209	5-Hydroxy methyl furfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	27.06	Anti-oxidant anti-proliferative activity
7	20.746	D-Glucitol, 1,4-anhydro	C <sub>18</sub> H <sub>34</sub> O <sub>6</sub>	346.459	27.81	Anti-bacterial
8	26.424	9,12-Octadecadienoic acid	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.47	3.53	Anticancer

**Table 6: Compounds identified by GC-MS in Hydroalcoholic extract of *Bauhinia variegata*.**

S.no	RT	Name	MF	M.W (g/mol)	Peak area %	Biological activity
1	4.896	2-Furanmethanol	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98.1	13.74	Antiviral activity
2	6.288	2(5H)-Furanone 5-methyl-(Identit)	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98.1	2.32	Antitumor activity
3	10.001	Diazene, bis (1,1-dimethyl ethyl)	C <sub>8</sub> H <sub>18</sub> N <sub>2</sub>	142.24	4.45	Anti-phytopathogenic
4	12.645	Benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	122.12	44.96	Antimicrobial, antiestrogenic, anti-inflammatory, anti-platelet aggregating, antiviral, anti-oxidant, antimutagenic, , antialgal,
5	13.048	5-Hydroxy methyl furfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	7.77	Anti-oxidant anti-proliferative activity
6	24.132	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45	3.27	Anti-oxidant, decrease blood cholesterol, anti-inflammatory
7	26.507	9-Octadecenoic acid (Z) methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	294.47	4.46	Anti-cancer

**Table 7: Compounds identified by GC-MS in Hydroalcoholic extract of *Caesalpinia bonducella*.**

S.no	RT	Name	MF	M.W (g/mol)	Peak area %	Biological activity
1	6.276	2(3H)-Furanone, 5-Methyl	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98.09	4.35	Antimicrobial activity
2	12.951	5-Hydroxy methyl furfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	4.46	Anti-oxidant anti-proliferative activity
3	26.438	9,12-Octadecadienoic acid methyl	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.47	4.74	Anticancer
4	33.496	9,12-Octadecadienoic acid (z,z)-2-hydroxy-1	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354.52	11.53	Anti-inflammatory, nematocide
5	37.191	Retinol, acetate	C <sub>22</sub> H <sub>32</sub> O <sub>2</sub>	328.5	4.00	Vitamin A
6	38.540	Rhodopin	C <sub>40</sub> H <sub>58</sub> O	554.9	5.10	Major compounds in phototrophic bacteria.

**Table 8: Compounds identified by GC-MS in Hydroalcoholic extract of *Saraca asoka*.**

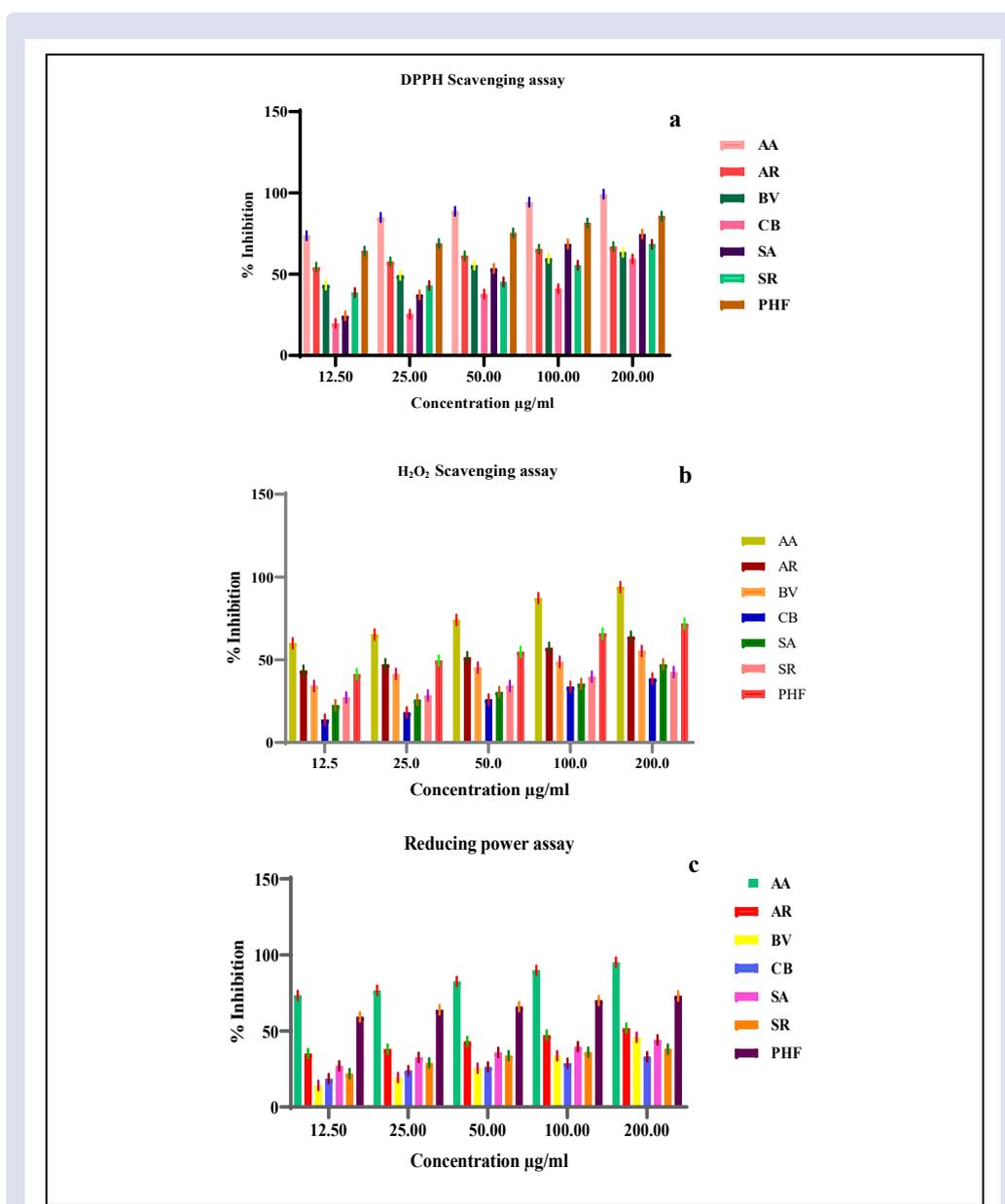
S.no	RT	Name	MF	M.W (g/mol)	Peak area %	Biological activity
1	4.453	Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	96.08	7.67	Antimicrobial activity
2	4.929	2-Furanmethanol	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98.1	4.99	Antiviral activity
3	9.584	Bicycle (2,2,1) Heptane-2-Carboxyl	C <sub>8</sub> H <sub>12</sub> O <sub>2</sub>	140.18	2.32	Antioxidant activity
4	12.164	Benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	122.12	2.64	Antifungal activity
5	13.362	5-Hydroxymethyl furfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	54.02	Anti-oxidant anti-proliferative activity
6	16.521	1,2,3-Benzenetriol	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126	6.37	Antimicrobial, Anti-inflammatory, Antioxidant, Analgesic, Insecticide, Anticancer, Cytotoxic

**Table 9: Compounds identified by GC-MS in Hydroalcoholic extract of *Symplocos racemosus*.**

S.no	RT	Name	MF	M.W (g/mol)	Peak area %	Biological activity
1	4.416	2-Furancarboxaldehyde	C <sub>5</sub> H <sub>5</sub> NO <sub>2</sub>	111.1	16.62	Antimicrobial
2	9.611	3,5-octadien-2-one	C <sub>8</sub> H <sub>12</sub> O	124.18	2.23	Antimicrobial
3	10.153	Levoglucosenone	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	3.75	Antioxidant
4	11.034	4H-Pyran-4-one, 2,3-dihydro 3,5-dihydroxy-6	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	144.12	2.58	Antimicrobial, anti-inflammatory, antiproliferative
5	13.300	5-Hydroxy methyl furfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	56.54	Anti-oxidant anti-proliferative activity
6	15.927	Phenol-4-Propyl	C <sub>9</sub> H <sub>12</sub> O	136.19	5.62	Antioxidant activity
1	4.416	2-Furancarboxaldehyde	C <sub>5</sub> H <sub>5</sub> NO <sub>2</sub>	111.1	16.62	Antimicrobial
2	9.611	3,5-octadien-2-one	C <sub>8</sub> H <sub>12</sub> O	124.18	2.23	Antimicrobial

**Table 10: Compounds identified by GC-MS in Hydroalcoholic extract of Polyherbal formulation.**

S.no	RT	Name	MF	M.W (g/mol)	Peak area %	Biological activity
1	4.933	2-Furanmethanol	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98.1	3.16	Antiviral activity
2	17.089	1-chlorodecane	C <sub>10</sub> H <sub>21</sub> Cl	176.72	45.35	Ontologies
3	20.500	Tetradecane 1-chloro	C <sub>14</sub> H <sub>29</sub> Cl	232.83	18.34	Antimicrobial activity
4	22.545	Methoxyacetic acid, pentadecyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>3</sub>	300.5	2.63	Antibacterial activity
5	24.136	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	2.96	Antioxidant, antibacterial, antifungal
6	24.676	1,2-benzenedicarboxylic acid	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	166.13	2.11	Antimicrobial, Antifouling, Anti-extended-spectrum beta-lactamase activity
7	26.433	9,12-octadecadienoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	2.64	Anti-cancer, antimicrobial activity



**Figure 3:** Antioxidant activity with DPPH (a), H<sub>2</sub>O<sub>2</sub> (b), and Reducing power assay (c) (AA-Ascorbic acid, AR-Asparagus racemosus, BV-Bauhinia variegata, CB-Caesalpinia bonducella, SA-Saraca asoka, SR-Symplocococcus racemosus, and PHF-Polyherbal formulation)

**Table 11: DPPH scavenging assay (in %).**

Conc. µg/ml	AA (std.)	AR	BV	CB	SA	SR	PHF
12.5	57.3 ± 0.789	54.51 ± 0.325	43.28 ± 0.286	19.58 ± 0.08	24.46 ± 0.721	38.53 ± 0.736	64.42 ± 0.283
25	64.73 ± 0.623	57.65 ± 0.786	49.24 ± 0.05	26.38 ± 1.073	37.53 ± 0.586	43.81 ± 0.987	69.02 ± 0.384
50	72.88 ± 0.928	61.41 ± 0.457	56.09 ± 0.609	37.47 ± 1.159	54.32 ± 0.644	44.81 ± 0.946	75.98 ± 0.605
100	85.13 ± 2.44	66.86 ± 1.11	60.7 ± 1.021	42.47 ± 1.22	68.54 ± 1.16	55.22 ± 0.575	81.62 ± 0.146
200	99.34 ± 0.185	65.15 ± 1.661	65.02 ± 1.362	60.57 ± 1.103	74.16 ± 0.575	68.81 ± 0.317	86.04 ± 0.442

Values were in mean ± standard deviation, n=3

**Table 12: Hydrogen peroxide assay (in %).**

Conc. µg/ml	AA (std.)	AR	BV	CB	SA	SR	PHF
12.5	59.98 ± 0.545	43.58 ± 0.545	34.69 ± 1.056	14.21 ± 0.638	22.96 ± 0.752	27.37 ± 0.495	42.02 ± 0.75
25	65.66 ± 0.929	47.28 ± 0.37	41.94 ± 0.645	17.99 ± 0.849	25.96 ± 0.939	28.48 ± 0.435	49.6 ± 0.125
50	74.87 ± 0.815	52.07 ± 0.676	46.02 ± 0.848	26.12 ± 0.305	31.4 ± 0.827	34.28 ± 0.561	55.067 ± 0.459
100	87.21 ± 0.27	57.16 ± 0.49	48.82 ± 0.845	33.85 ± 0.297	35.58 ± 0.511	39.83 ± 0.34	65.85 ± 0.58
200	93.97 ± 0.73	63.96 ± 0.07	55.84 ± 0.861	38.75 ± 0.265	47.21 ± 0.696	42.84 ± 0.27	72.12 ± 0.694

Values were in mean ± standard deviation, n=3

**Table 13: Reducing power assay (in %).**

Conc. µg/ml	AA (std.)	AR	BV	CB	SA	SR	PHF
12.5	73.25 ± 0.451	35.13 ± 0.141	14.17 ± 0.775	18.55 ± 0.236	26.79 ± 0.767	21.83 ± 0.564	59.2 ± 0.235
25	76.69 ± 1.039	37.94 ± 0.207	19.41 ± 0.223	23.93 ± 0.679	32.67 ± 0.679	29.04 ± 0.103	63.86 ± 0.869
50	82.57 ± 0.38	43.1 ± 0.478	25.48 ± 0.373	26.35 ± 0.58	35.95 ± 0.842	33.73 ± 0.584	65.97 ± 0.512
100	89.8 ± 0.68	47.23 ± 0.5	33.66 ± 0.608	28.77 ± 0.911	39.7 ± 0.634	35.97 ± 0.43	70.08 ± 0.61
200	95.2 ± 0.556	51.82 ± 0.345	45.76 ± 0.723	33.09 ± 0.404	43.92 ± 0.369	37.63 ± 0.631	73.02 ± 0.881

Values were in mean ± standard deviation, n=3

PHF was compared to ascorbic acid it as mention in Table 12 and Figure 3 (c). Selected medicinal plants and PHF were compared to the ascorbic acid standard it gives good scavenging inhibition against free radicals it as shown in Figure 3. In this activity hydroalcoholic extract of all the selected medicinal plants and its polyherbal formulation showed unresolved scavenging properties on DPPH, reducing power assay, and H<sub>2</sub>O<sub>2</sub> radical activities. In accumulation, TPC and TFC of the individual plant extracts and PHF were evaluated. It was detected that individual plants extract and PHF confined rich source of flavonoid and phenolic content that capacity has reported for the strong antioxidant activity detected against the free radicals. These results exposed that selected medicinal plant extracts and polyherbal formulation have various phytochemical constituents which might be for many pharmacological activities. Nevertheless, because of the above-introduced results, selected medicinal plants and their PHF cloud be explored as a potential new wellspring of natural antioxidants in the food, nutraceuticals, pharmaceutical and cosmetic industry.<sup>28</sup>

## CONCLUSION

There is a requirement for a time about the logical assessment of novel polyherbal formulation for upcoming generations. The improvement of new polyherbal formulation has been prosperous after a wide literature review. Standardization of herbal medication is a vital significance in initiating its proper identity, purity, quality, and therapeutic efficacy. The macroscopic, physicochemical, qualitative, and quantitative phytochemical analyses are the confirmatory tests for standardization and quality control. Phytochemical screening revealed the presence of various constituents and estimation of bioactive compounds confirmed the high concentration of all the bioactive contents in all the individual plant extract when compared to the polyherbal formulation. Antioxidant activity of all the individual extract and polyherbal formulation showed the highest scavenging activities against DPPH, radical scavenging, and hydrogen peroxide assay. A GC-MS result of all the individual extract and polyherbal formulation contains various bioactive components and it's suggested as a PHF of phytopharmaceutical importance. In

conclusion, the present study can be used as reference information for proper identification, authentication, and *in vitro* antioxidant assay of a novel PHF can be explored for its applications in the prevention of free radical related disease.

## CONFLICTS OF INTEREST

None.

## ABBREVIATIONS

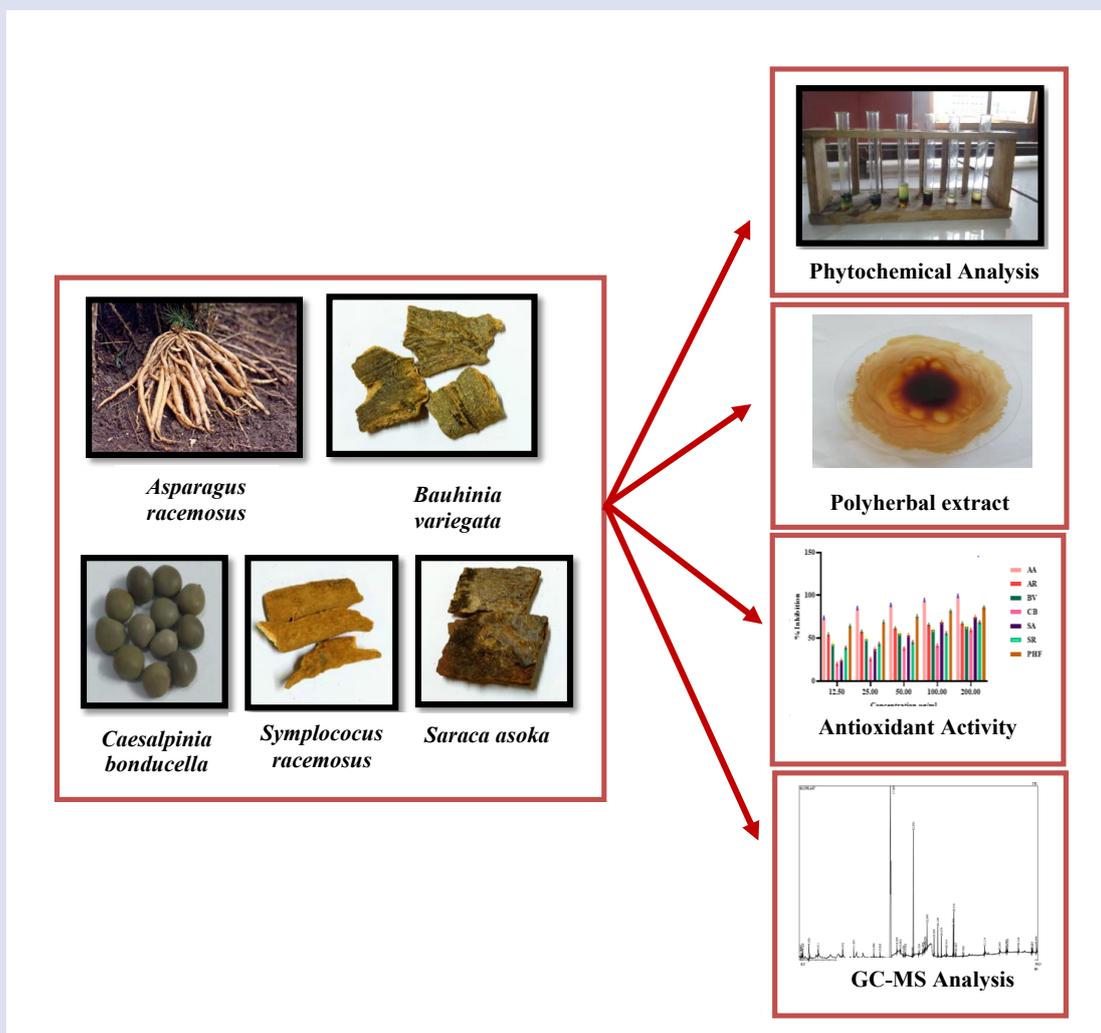
A. racemosus and AR – *Asparagus racemosus*; B. variegata and BV – *Bauhinia variegata*; C. bonducella and CB – *Caesalpinia bonducella*; S. asoka and SA – *Saraca asoka*; S. racemosus and SR – *Symplocococcus racemosus*; PHF – Polyherbal formulation; TAC: Total alkaloid content; TFC: Total flavonoid content; TGC: Total glycoside content; Total steroidal content; TPC: Total phenolic content; DPPH -2, 2 – diphenyl – 1picryl hydrazyl; IC<sub>50</sub>: Concentration of samples resulting in 50% inhibition; GAE: Gallic acid equivalent, std.: Standard; H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide; GC-MS: Gas chromatography – Mass spectroscopy.

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## GRAPHICAL ABSTRACT



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