

# ***In-vitro* qualitative and quantitative analysis of certain nutraceuticals as diuretic and antioxidant for hepatobiliary disorders (HBD)**

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## **Abstract**

Nutraceuticals are plant, dietary/food and animal origin, safe and eco friendly, its having potent curative and preventing certain diseases conditions. Hence, the study was commenced with the objectives of an *in-vitro* qualitative and quantitative analysis of certain nutraceuticals as diuretic and antioxidant potential for HBD. Different parts (leaves, root, stem, seeds and whole plant) of plants *viz. Phyllanthus niruri, Solanum nigrum, Boerhavia diffusa, Eclipta alba, Macrotyloma uniflorum, Tinospora cordifolia* and *Murraya koenigii* were collected and aqueous, ethanolic and 50% ethanolic extract was prepared. *In-vitro* qualitative phytochemical screening of different herbal extracts showed best in 50% ethanolic extracts of *Murraya koenigii* leaves, *Eclipta alba* whole plant, *Phyllanthus niruri* whole plant, *Solanum nigrum* whole plant, *Macrotyloma uniflorum* seeds, and *Boerhavia diffusa* whole plant. *In-vitro* quantitative analysis 50% ethanolic extracts of *Murraya koenigii* leaves, *Eclipta alba* whole plant, *Phyllanthus niruri* whole plant, *Solanum nigrum* whole plant, *Macrotyloma uniflorum* seeds, and *Boerhavia diffusa* whole plant. 50% ethanolic extracts of *Phyllanthus niruri* whole plant and *Eclipta alba* whole plant followed by *Murraya koenigii* leaves, *Macrotyloma uniflorum* seed and *Boerhavia diffusa* whole plant were noticed better diuretic and antioxidant property by *In-vitro* qualitative and quantitative phytochemical analysis. The study was concluded that the combination or mixture of these extracts with appropriate proportion could have effective diuretic and antioxidant potentials against hepatobiliary disorders.

**Keywords:** *Boerhavia diffusa, Eclipta alba*, hepatobiliary disorders, *Macrotyloma uniflorum, Murraya koenigii*, Nutraceuticals, *Phyllanthus niruri, Solanum nigrum, Tinospora cordifolia*

## **Introduction**

Liver plays a pivotal role in the regulation of body metabolism, secretion and detoxification process of many compounds. Hepatic failure or hepatobiliary disorders (HBD) by drug toxicity, cholestasis, hepatic vein thrombosis and liver tissue necrosis are the major symptoms. Chronic hepatic disease damages the liver leading to hepatic fibrosis and cirrhosis followed by ascites. For effective treatment of hepatobiliary diseases, therapy requires disease directed interventions with the aim to eliminate the causative factors by reducing hepatic inflammation, minimizing fibrosis, controlling complications and initiating hepatic regeneration. Ascites is the common complication of chronic liver diseases and there were no effective therapeutic protocol for complete curative. Extensive literature has been published on ascites of hepatobiliary dysfunctions and their remedies, but some of the ascites condition are failed to respond diuretics because of high level of serum aldosterone in many cirrhotic conditions<sup>1</sup>. Normally body posses defence mechanism against free radicals induced oxidative stress, which involve preventive mechanism, repair mechanism, physical defence and antioxidant mechanisms<sup>2</sup>. These defence mechanism were down by chronic infection of the host. In this way medicinal plants are very much useful to treat chronic diseases without any side effects and betterment to prolong the lifespan of patients.

Medicinal plants play a key role in human health care. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems. In spite of the availability of more than 300 preparations for the treatment of jaundice and chronic liver diseases in Indian system of medicine (using more than 87 Indian medicinal plants) only four terrestrial plants have been scientifically elucidated while adhering to the internationally acceptable scientific protocols<sup>3,4</sup>.

Dietary supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods<sup>5</sup> *i.e.* Nutraceuticals. Many nutraceuticals of animal or plant origin have potent antioxidant properties<sup>6</sup>. Dearing *et al.*<sup>7</sup> suggested that the plants/herbs/food materials which possess the compound of terpenes, phenolics and alkaloid are very good diuretic effects. Antioxidant scavenges free radicals in the body and prevents tissue damage. Therefore, nutraceuticals supplement may protect cellular structures against oxidative stress and may catalyze the effectiveness of standard therapeutic regimen against ascites due to liver origin and may take shorter duration with better recovery. The present study may find out the better nutraceutical composite for the treatment of HBD. Hence, the study was initiated with the objectives of an *in-vitro* qualitative and quantitative analysis of certain nutraceuticals as diuretic and antioxidant for HBD.

### Materials and Methods

Chemical compounds having diuretic and antioxidant property of plants and or plant origin were screened for their *in vitro* diuretic and antioxidant potential. Screening was done on the basis of available scientific literature and indigenous technical knowledge of common people. Bio-organics of plant origin were identified by their local as well as scientific name.

#### Collection plants

Herbs/ plants materials were collected from in and around Bareilly. Different parts (leaves, root, stem, seeds and whole plant) of following plants *Phyllanthus niruri*, *Solanum nigrum*, *Boerhavia diffusa*, *Eclipta alba*, *Macrotyloma uniflorum* seed, *Tinospora cordifolia* and *Murraya koenigii* were collected.

**Preparation of plant extracts:** Selected plants (leaf, stem, root, seeds and whole plant) were cleaned and shed dried and meshed into coarse powder by mechanical grinder. All the materials were subjected to extraction by aqueous, ethanolic and 50% ethanolic (50% aqueous+ 50% ethanol) by Soxhlet method at temperature of 40-41°C with standard protocol. 100 g of each dried samples was taken for extraction and yield of extract was recorded per 100 g of sample. Collected extracts were air dried and stored in deep freezer for further analysis.

**Test for alkaloids:** Evaporated the aqueous, alcohol and chloroform extract separately and added diluted HCl to the residue. Mixed well and followed by filtrated collection was added with the following reagents.

a) **Dragendorff test:** To 2-3ml of filtrate added few drops of Dragendorff reagent - orange brown precipitate indicated the presence of alkaloids<sup>8</sup>.

b) **Mayer's test:** To 2- 3 ml of filtrate added few drops of Dragendorff reagent - Yellowish white precipitate indicated the presence of alkaloids<sup>9</sup>.

**Test for phenolic compound:** with 2- 3ml of aqueous and alcohol extracts, added few drops of following reagents<sup>8</sup>.

a) 5 % FeCl<sub>3</sub> solution: deep black color for positive sample.

b) Lead acetate solution: red color for positive sample.

**Test for terpenes:** 0.5 g of test powder/ solution dissolved in 5 ml of methanol. 2 ml of filtrate were treated with 1 ml of 2, 4- dinitrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow- orange color observed as an indication of terpenoids<sup>10</sup>.

#### Test for Flavonoides

a) **Shinoda test:** To dry powder of extract, add 5ml of 95% ethanol followed by few drops of con HCl and 0.5 g magnesium turnings- Pink color indicate flavonoides<sup>8</sup>.

b) Added lead acetate solution to dry powder of extract- Yellow colored precipitate indicates presence of flavonoides<sup>8</sup>.

**Ammonium vandate test:** Dissolved 1.62 g of anhydrous ammonium vandate in 125 ml of concentrated sulphuric acid, cooled and added into 125 ml of ice-cold water. After dilution of 10 fold, added few drops to the extract and the presence of brick red / orange to yellow/ green color indicates presences of alkaloids, phenol and steroid compound<sup>8</sup>.

#### *In-vitro* quantitative analysis for antioxidant potentials

**Ferric reducing antioxidant power assay (FRAP):** The FRAP assay is versatile and can be readily applied to various extracts of plants. In this assay, the antioxidant activity was determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron. The FRAP assay was carried out according to the procedure of Sahgal

*et al.*<sup>11</sup>. The FRAP reagent was prepared by mixing acetate buffer (25 ml, 300 mmol/L, pH 3.6), 10 mmol/L TPTZ solution (2.5 ml) in 40 mmol/L HCl and 20 mmol/L FeCl<sub>3</sub> solution (2.5 mL) in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared freshly and warmed to 37°C in a water bath prior to use. Extract sample (150µl) was added to the FRAP reagent (4.5 mL) and the absorbance of the reaction mixture was recorded at 593 nm after 4 min. The assay was carried out in triplicates for accuracy. The standard curve was constructed using FeSO<sub>4</sub> solution (0.5-10 mg/mL). The results were expressed as µmol Fe (II)/g dry weight of plant extracts. L-ascorbic acid was also used as a comparative model for this assay.

**Ascorbate – iron (III) – catalyzed phospholipid peroxidation:** The ability of the extracts and the potencies to scavenge hydroxyl radicals was determined by the modified method of Aruoma *et al.*<sup>12</sup>. Goat liver was mixed (1:10) with 10 mM phosphate-buffered saline (PBS, pH 7.4) and sonicated in an ice bath for preparation of the homogenate liposomes. The liposomes (0.2 ml) were added with 0.5 ml of PBS buffer, 0.1 ml of 1 mM FeCl<sub>3</sub> and various volumes (100µl and 200µl) of plant extracts and subsequently 0.1 ml of 1 mM ascorbic acid was added. After incubation at 37°C for 60 min, 1 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 2000 rpm for 10 min at room temperature. Finally, one ml of 0.67% 2-thiobarbituric acid (TBA) in 0.05 M NaOH was added to the supernatant, vortexed and heated in a water bath at 100°C for 20 min. After cooling, 1 ml of distilled water was added and absorbance was recorded at 532 nm. Control containing all reagents except the extracts. The assay was carried out in triplicate for accuracy. Vitamin E was also used as a comparative model for this assay. The percentage inhibition activity was calculated as: [(Abs. of control – Abs. of sample)/Abs. of control] × 100%.

**Total flavonoides content:** The total flavonoides content of the extracts and the potencies was determined according to colorimetric method as described by Nabavi *et al.*<sup>13</sup>. In brief, the sample solution (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% NaNO<sub>2</sub> solution (0.15 ml). After 6 min of incubation, 10% AlCl<sub>3</sub> solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 4% NaOH solution (2 ml) to the mixture. Consequently, water was added to the sample to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm. The total flavonoid content was expressed in mg of catechin per gram of extract.

**Total phenol content:** The total phenolic content in the extracts and the potencies was measured using Folin-Ciocalteu reagent method Biglari *et al.*<sup>14</sup>. The samples (0.4 mL) (1mg/mL extracts) were transferred into test tubes. To this solution, distilled water (1.0 mL) and Folin-Ciocalteu reagent (1.0 mL) were added, and the tubes shaken thoroughly. After 1 min, sodium carbonate solution (Na<sub>2</sub>CO<sub>3</sub>, 1.6 mL, 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. A linear dose response regression curve was generated using absorbance reading of gallic acid at the wavelength of 765 nm using UV spectrophotometer. The total phenolic compounds concentration in the extract and potencies was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g). The content of phenolic compounds in the extract and the potency was calculated using this formula: C = A/B; where C is expressed as mg GAE/g dry weight of the extract; A is the equivalent concentration of gallic acid established from calibration curve (mg) and B is the dry weight of the extract (g).

**Determination of scavenging activity against hydrogen peroxide:** The free radical scavenging activity of plant extract against hydrogen peroxide was determined using the method of Ruch *et al.*<sup>15</sup>. Samples with various concentrations were added to 0.1 M phosphate buffer solution (pH 7.4, 3.4 ml) and mixed with 43 mM hydrogen peroxide solution (0.6 ml). After 10 min, the reaction mixture absorbance was determined at 230 nm. The reaction mixture without sample was used as the blank. Ascorbic acid was used as a reference compound<sup>16</sup>. The percentage inhibition activity was calculated as: [(Abs. of control – Abs. of sample) /Abs. of control] × 100%.

**Statistical Analysis:** Values were expressed as Mean ± SE. Data were analyzed by one-way and P < 0.05 is considered to be a statically significant.

## Results

*In-vitro* test performed with the extracts prepared by aqueous, ethanolic and 50% ethanolic solvent method. The results of *in-vitro* test has been graded as negative (-), positive (+) and highly positive (++)

**Test for alkaloids:** Highly positive (++) alkaloids were noticed in 50% ethanolic extracts of *Murraya koenigii* leaves, *Phyllanthus niruri* whole plant and *Solanum nigrum* whole plant.

**Test for Phenol:** Highly positive (++) phenol was noticed in 50% ethanolic extracts of *Boerhavia diffusa* whole plant, *Eclipta alba* whole plant, *Macrotyloma uniflorum* seeds, *Phyllanthus niruri* whole plant and aqueous extract of *Eclipta alba* whole plant as per 5% FeCl<sub>2</sub> method, whereas 50% ethanolic extracts of *Eclipta alba* whole plant and *Phyllanthus niruri* whole plant in lead acetate method.

**Test for Terpenes:** Highly positive (++) terpenes was noticed in 50% ethanolic extracts of *Eclipta alba* whole plant and *Murraya koenigii* leaves

**Ammonium vandate test:** Positive for (+) flavonoides, phenol and steroids were noticed in 50% ethanolic extracts of *Solanum nigrum*, *Phyllanthus niruri* and *Murraya koenigii* leaves.

**Test for flavonoides:** Highly positive (++) flavonoid was noticed in 50% ethanolic extracts of *Boerhavia diffusa* whole plant, *Eclipta alba* whole plant, *Murraya koenigii* leaves and *Macrotyloma uniflorum* seeds.

Critical analysis of *in-vitro* qualitative phytochemical analysis of different herb extracts revealed best in 50% ethanolic extracts of *Murraya koenigii* leaves, *Eclipta alba* whole plant, *Phyllanthus niruri* whole plant, *Solanum nigrum* whole plant, *Macrotyloma uniflorum* seeds, and *Boerhavia diffusa* whole plant.

#### ***In-vitro* quantitative analysis for antioxidant and hepatoprotective activity**

*In-vitro* quantitative test performed with the plant extracts which revealed highly positive (++) results as of during *in-vitro* qualitative analysis.

**Ferric Reducing Antioxidant Power Assay (FRAP):** The FRAP assay was applied to 50% ethanolic extracts of various plants. In this assay, antioxidant activity was determined based on the ability to reduce ferric (III) iron to ferrous (II) iron. The standard curve was generated in the range of 0.25mg/ml, 0.5mg/ml and 1.0mg/ml of ferrous sulphate and the results were expressed as mmol ferrous ion equivalent per gram of sample dry weight ( $y = 0.1206x - 0.01$ ,  $R^2 = 0.9989$ ). FRAP value revealed significant ( $p < 0.05$ ) increase level of 50% ethanolic extracts of *Phyllanthus niruri* whole plant and *Eclipta alba* whole plant among seven plants evaluated.

**Ascorbate – Iron (III) – Catalyzed phospholipid peroxidation (AICPP):** Critical analysis revealed higher activity of AICPP in 50% ethanolic extracts of *Eclipta alba* whole plant to scavenge hydroxyl radicals generated by ascorbic –iron III to inhibit the formation of 2-thiobarbituric acid reactive species (TRABS) though was not significantly different from standard. However, significantly ( $p < 0.05$ ) reduced AICPP in *Macrotyloma uniflorum* seeds and *Solanum nigrum* whole plant were noticed.

**Total flavonoides content (TFC):** The total flavonoid content in the 50% ethanolic extracts were expressed as mg GAE/g dry weight of the extract is reported as catechin equivalents by reference to standard curve ( $y = 0.0089x + 0.0165$ ,  $R^2 = 0.9276$ ). The presence of catechin content of 50% ethanolic extracts of different plant extracts revealed significant ( $p < 0.05$ ) increased catechin content in 50% ethanolic extract of *Phyllanthus niruri* whole plant, *Macrotyloma uniflorum* seeds and *Murraya koenigii* (leaves).

**Total phenol content (TPC):** The content of phenolic compounds in the extract was expressed as mg GAE/g dry weight of the extract. Total phenol content was determined by Folin Ciocalteu method and reported as gallic acid equivalents by reference to standard curve ( $y = 0.0451x - 0.006$ ,  $R^2 = 0.983$ ). Total phenolic content was significantly ( $p < 0.05$ ) increased in 50% ethanolic extracts of *Phyllanthus niruri* whole plant followed by *Murraya koenigii* leave and *Solanum nigrum* whole plant.

**Determination of Scavenging Activity against Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>):** Scavenging activity of 50% ethanolic extracts against hydrogen peroxide were significantly ( $p < 0.05$ ), higher in *Eclipta alba* whole plant followed by *Boerhavia diffusa* whole plant and *Macrotyloma uniflorum* seeds.

Critical analysis of *in-vitro* quantitative analysis of antioxidant and hepatoprotective activity of different herbs/plant material depicted highest activity in 50% ethanolic extracts of *Phyllanthus niruri* whole plant, *Eclipta alba* whole plant followed by *Macrotyloma uniflorum* seed, *Solanum nigrum* whole plant, *Murraya koenigii* leaves, and *Boerhavia diffusa* whole plant.

### **Discussion**

Plant materials containing high phenolic, alkaloids and terpenes are considered as the predominant component for exerting diuretic activity<sup>7</sup>. Many nutraceuticals of animal or plant origin have potent antioxidant properties<sup>6</sup>. Antioxidants are vital substances which possess the ability to protect body from damage by free radical induced oxidative stress<sup>17,18</sup>. Therefore in the present study plants were selected having potent diuretic activity and antioxidant property based on *in-vitro* qualitative and quantitative analysis.

The presence of alkaloids, flavonoids, tannins, saponins and terpenoids shows hepatoprotection by inhibiting the free radicals mediated damage<sup>19,20</sup> and diuretic<sup>7</sup> claimed that flavonoids, triterpens and tannin were antioxidant agent and may interfere with free radicals formation. Similar findings were also recorded with *Eclipta alba*<sup>21,22,23</sup>, *Solanum nigrum*<sup>24-27</sup>, *Macrotyloma uniflorum*<sup>28,29</sup>, *Murraya koenigii*<sup>30,31</sup>, *Phyllanthus niruri*<sup>32,33</sup> and *Boerhavia diffusa*<sup>34,35</sup>.

Antioxidant activity is determined based on the ability to reduce ferric (III) iron to ferrous (II) iron<sup>11</sup>. 50% ethanolic extract revealed good ability to reduce ferric (III) iron to ferrous (II) iron. Significantly higher activity with 50% ethanolic extract of *Eclipta alba* whole plant to scavenge hydroxyl radicals generated by ascorbic –iron III to inhibit the formation of 2-thiobarbituric acid reactive species (TRABS) were observed during present study. Phospholipids are being regarded as valuable substrate for the appraisal of antioxidant potential and it is being considered as an ideal model to study dietary components and drug for membrane lipid peroxidation<sup>36</sup>. Fe (III) to Fe II reduction and synthetic radical scavenging has been considered to indicate potential antioxidant activity<sup>37</sup>. In the presence of iron (III) and ascorbic acid, phospholipid based liposome rapidly

undergo hydroxyl radical mediated peroxidation producing malondialdehyde as associated aldehydes<sup>38</sup>. The phenolic compounds present in the extract may contribute directly to the antioxidative action of the plant suggesting that the polyphenols present in the extract could be responsible for its beneficial effects<sup>39</sup>. Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids and tannins) have diverse biological effects such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects, as a result of their antioxidant activity<sup>40</sup>. Scavenging activity of 50% ethanolic extracts against hydrogen peroxide were significantly higher in *Eclipta alba* whole plant, *Boerhavia diffusa* whole plant and *Macrotyloma uniflorum* seeds. Addition of H<sub>2</sub>O<sub>2</sub> to extract can lead to transition metal ion dependent OH mediated oxidative DNA damage. Levels of H<sub>2</sub>O<sub>2</sub> at or below about 20-50 mg seem to have limited cytotoxicity to many cell types<sup>41</sup>. Since phenolic compounds present in the plant extract are good electron donors, they can accelerate the conversion of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O<sup>15</sup>.

### Conclusions

*In-vitro* qualitative and quantitative phytochemical analysis of various parts of herbs with different solvent extract were observed better diuretic and antioxidant property in 50% ethanolic extracts of *Phyllanthus niruri* whole plant and *Eclipta alba* whole plant followed by *Murraya koenigii* leaves, *Macrotyloma uniflorum* seed and *Boerhavia diffusa* whole plant. Hence, the study was concluded that the combination or mixture of these extracts with appropriate proportion could have effective diuretic and antioxidant potentials against hepatobiliary disorders.

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Table 1. In-vitro qualitative analysis of different herbal extracts

S. No	Plant extracts	Yield /100g of extract	Alkaloids		Phenols		Terpenes	Amino acids	Flavonoids
			Magnesium	Drugs	5% Fecul	Leads			
1	<i>Murraya koenigii</i> (leaves)-50% ethanol	13.0	+	+	+	+	++	+	++
2	<i>Murraya koenigii</i> (leaves)-aqueous	30.28	-	-	+	+	+	+	+
3	<i>Murraya koenigii</i> (leaves)-ethanol	8.44	-	-	-	-	-	-	-
4	<i>Eclipta alba</i> whole plant- 50% ethanol	30.42	-	-	++	++	++	-	+
5	<i>Eclipta alba</i> whole plant-aqueous	20.44	-	-	++	+	+	-	+
6	<i>Eclipta alba</i> whole plant-ethanol	13.34	-	-	+	+	+	-	+
7	<i>Macrotyloma uniflorum</i> -50% ethanol	16.95	+	+	++	+	+	-	++
8	<i>Macrotyloma uniflorum</i> - aqueous	13.5	+	-	+	+	-	-	-
9	<i>Macrotyloma uniflorum</i> - ethanol	4.05	+	-	+	+	-	+	+
10	<i>Phyllanthus niruri</i> leaves-50% ethanol	28.42	+	+	+	+	+	+	-
11	<i>Phyllanthus niruri</i> leaves-aqueous	22.81	-	-	+	+	+	-	+
12	<i>Phyllanthus niruri</i> leaves-ethanol	19.52	-	-	+	+	-	-	-
13	<i>Phyllanthus niruri</i> whole	4.90	-	-	+	+	-	-	-

plant- ethanol										
14	<i>Phyllanthus niruri</i>	whole plant- 50% ethanol	18.0	+	+	++	+	+	+	-
15	<i>Phyllanthus niruri</i>	whole plant- aqueous	22.06	-	-	+	+	+	-	+
16	<i>Boerhavia diffusa</i>	root- 50% ethanol	18.28	+	-	+	+	+	-	-
17	<i>Boerhavia diffusa</i>	root- ethanol	3.52	-	-	-	-	-	-	-
18	<i>Boerhavia diffusa</i>	(whole plant)-50% ethanol	21.62	+	-	++	+	+	-	++
19	<i>Boerhavia diffusa</i>	(whole plant)-aqueous	26.5	-	-	+	+	+	-	-
20	<i>Boerhavia diffusa</i>	(whole plant)-ethanol	15.56	-	-	+	-	-	-	+
21	<i>Solanum nigrum</i>	fruit - ethanol	2.86	+	-	+	-	+	+	+
22	<i>Solanum nigrum</i>	fruit-50% ethanol	19.0	+	-	+	+	+	-	+
23	<i>Solanum nigrum</i>	fruit- aqueous	39.5	+	-	+	+	+	+	+
24	<i>Solanum nigrum</i>	whole plant- 50% ethanol	12.68	+	-	+	+	+	+	+
25	<i>Solanum nigrum</i>	whole plant- aqueous	33.64	+	-	+	+	-	+	+
26	<i>Solanum nigrum</i>	whole plant- ethanol	27.82	+	+	+	+	+	+	+
27	<i>Tinospora cordifolia</i>	-50% ethanol	2.42	-	-	+	+	+	-	+
28	<i>Tinospora cordifolia</i>	- ethanol	1.94	-	-	+	-	-	-	+

Table 2. In-vitro quantitative analysis of different herbal extracts

S.No	Plants/ Tests	AICPP (% inhibition)	TPC (GAE/g)	FRAP (mmol Fe II/g)	TFC (mg catechin/g)	H <sub>2</sub> O <sub>2</sub> (% inhibition)
1.	<i>Eclipta alba</i> (whole plant)-50% ethanol	43.78±0.90 <sup>b</sup>	9.36±0.88 <sup>ab</sup>	0.51±0.07 <sup>ab</sup>	5.22±0.21 <sup>b</sup>	86.09±0.89 <sup>ab</sup>
2.	<i>Solanum nigrum</i> (whole plant)- 50% ethanol	15.26±0.58 <sup>a</sup>	13.13±0.44 <sup>b</sup>	0.42±0.09 <sup>a</sup>	6.09±0.41 <sup>b</sup>	70.28±0.62 <sup>abc</sup>
3.	<i>Macrotyloma uniflorum</i> - 50% ethanol	11.65±0.13 <sup>a</sup>	7.44±0.67 <sup>a</sup>	0.29±0.04 <sup>a</sup>	7.13±0.84 <sup>bc</sup>	72.41±0.56 <sup>abc</sup>
4.	<i>Murraya koenigii</i> (leaves)- 50% ethanol	10.04±0.74 <sup>a</sup>	15.53±0.05 <sup>b</sup>	0.41±0.08 <sup>a</sup>	6.09±0.05 <sup>b</sup>	57.05±0.35 <sup>ab</sup>
5.	<i>Phyllanthus niruri</i> (whole plant) - 50% ethanol	8.43±0.09 <sup>a</sup>	23.70±0.69 <sup>d</sup>	1.30±0.33 <sup>bc</sup>	13.50±0.34 <sup>d</sup>	52.63±0.36 <sup>a</sup>
6.	<i>Boerhavia diffusa</i> (whole plant)- 50% ethanol	2.81±0.06 <sup>a</sup>	6.72±0.65 <sup>a</sup>	0.24±0.0 <sup>a</sup>	1.55±0.83 <sup>a</sup>	76.92±0.99 <sup>abc</sup>
7.	Standard	39.45±0.46 <sup>b</sup>		0.58±0.07 <sup>a</sup>		58.43±0.45 <sup>ab</sup>