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PHARMACOGNOSTICAL EVALUATION AND ANTIOXIDANT EFFECT OF SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT: Since ancient times, herbal medicines have been utilized globally to prevent and cure a wide range of medical disorders. Despite the fact that they are employed in many different medical systems, it remains a challenge to ensure their quality control, proper scientific validation and standardization. Systematic validation and scientific regulation of natural formulations are required for the future improvement of traditional systems of medicine. The herbal products produced with proper standardization and validation are often more efficient and have fewer adverse effects. In the present study, the stem bark of *Ficus religiosa*, stems of *Tinospora cordifolia*, leaves of *Moringa olifera* and roots of *Boerhaavia diffusa* have been selected. Due to pharmacological effects present in these plants, they are widely used in different traditional medicines. All the chosen plants are standardized by powder microscopy, physicochemical parameters, phytochemical screening, heavy metal testing *etc.* Comparative analysis of these parameters helped detect adulteration in each of these selected plants. Further, the comparative study of *in-vitro* antioxidant profiles of all these selected plants helped develop the herbal formulation for treating diseased conditions.

INTRODUCTION: The environment in which we live, is going to govern the health of people. For the good health of people, they want healthy surroundings and a good health care system that should be cost-effective and available to everyone. Advancements in the modern health care system have discovered several synthetic medicines which have helped a lot in diagnosing, preventing and treating different diseases ^{1,2}.

These synthetic drugs can produce life-threatening side effects if given in a high dose or used for a long duration ^{3, 4}. Globally, the population is shifting towards traditional medicine systems like Ayurveda, Siddha, Unani, Homeopathy, *etc.* Traditional medicines were the backbone for treating diseases in India during ancient times before allopathic medicines were introduced ⁵.

In several surveys conducted by different organizations in developing countries, around 80% of the population relies on herbal products for healthy living. A large proportion of the worldwide medicine market is based on herbal medicines, which are utilized as over-the-counter products, raw materials for pharmaceuticals and own-

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prescribed formulations of plant origin^{6,7}. Despite this, the main disadvantage of herbal products is their inconsistent and variable pharmacological effects. Consequently, herbal products could only be designed as rational drugs per their quality parameters. To obtain consistent pharmacological effects, toxic effects, and clinical results, their constituents need to be well documented^{9,10}. In the present study, a few Indian medicinal plants are selected to study quality parameters for standardization. All these plants are used in different herbal formulations due to their various therapeutic effects in traditional or folk medicines. The selected plants and parts are the stem bark of *Ficus religiosa*, the stem of *Tinospora cordifolia*, the leaves of *Moringa oleifera*, and the roots of *Boerhaavia diffusa*¹¹.

Ficus religiosa Linn. (Moraceae) is called “Pipal tree” in Hindi. It is considered a religious tree for communities like Hindus, Buddhists, etc. This plant is mentioned as “Asvattha” in Ayurveda. It has medicinal value in the Indian traditional and folk medicine system^{12,14}. *Tinospora cordifolia* (Menispermaceae) called “Giloy” in Hindi, is a perennial climber shrub. It is abundantly found in India, Sri Lanka, China and Myanmar¹⁶. It is termed as “Rasayana” due to its significant use in traditional medicine¹⁷.

Moringa oleifera (Moringaceae) is widely distributed throughout the world¹⁸. Almost every part of the plant has been used for various diseases in the traditional medicine system of South Asia¹⁹. *Boerhaavia diffusa* Linn (Nyctaginaceae), is known as Punarnava in Sanskrit and Hogweed in English. It is native to India and a perennial creeping weed or ascending herb²⁰.

Morphological Characters and Phytoconstituents: The bark of *Ficus religiosa* is flat or slightly curved; the outer bark is greyish or ash-colored, exfoliated with irregularly rounded flakes of 2–2.5cm thickness. The bark is odorless, and its taste is astringent. The bark contains phytosterols like lanosterol, β -sitosterol, glucoside (β -sitosteryl-d-glucoside), and stigmasterol. The bark of *Ficus religiosa* comprises around 8.7% of the total tannin content, and other phytoconstituents are vitamin K1, n-octacosanol, methyl oleonate and lupen-3-one. Bergapten and Bergaptol are substituted

furanocoumarins^{21,22}. The stem of *Tinospora cordifolia* is filiform and climbs in nature²³. The powder of the stem is light brown or greenish brown in color with a characteristic smell and bitter taste. The chemical constituents of stem belong to different classes like alkaloids, glycosides, steroids, phenolics, aliphatic compounds and polysaccharide^{24,25}. The phytoconstituents isolated from the stem are cordifolioside A & B, berberine, 1,2-Substituted pyrrolidine, amritoside A, B, C and D, octacosanol, etc.^{26,28}. The leaves of *Moringa oleifera* are pale green in color, compound, and alternate with a characteristic odor and bitter taste²⁹. Leaves are rich in vitamins, carotenoids, flavonoids, and polyphenols. It contains phytoconstituents like kaempferitrin, isoquercetin, rhamnetin, kaempferol and quercetin, zeatin, ascorbic acid, phenolic, flavonoids, vitamin E^{30,33}.

The roots of *Boerhaavia diffusa* are cylindrical, light brown with a rough surface³⁴. It contains boeravinones A-1, B-1, C-2, D, E and F, behenic acid, beta-sitosterol, boerhaavicacid, borhavine, borhavone, campesterol, daucosterol, beta-ecdysone, hentriacontane N, hypoxanthine-9-l-arabinofuranoside, ursolic acid and 5,7-dihydroxy-3,4-dimethoxy-6,8-dimethyl flavones³⁵.

Traditional uses of Selected Plants: In traditional medicine system, *Ficus religiosa* stem bark extract is used to treat pain, neurodegeneration, ulcer, hepatic disorders, kidney disorders, diarrhoea, different microbial infections, diabetes, and other conditions. This extract also has antiseptic and astringent properties^{36,38}. In the ayurvedic medicine system, *Tinospora cordifolia* is used to treat several diseases like jaundice, weakness, blood disorders, fever, viral hepatitis, inflammation, microbial diseases, dyspepsia, urinary diseases, skin diseases, allergic conditions, diabetes, etc. It is also used as an anticancer, antistress, and immunomodulator^{39,42}. *Moringa oleifera* possesses several pharmacological activities such as analgesic, antifungal, antibacterial, antihypertensive and antispasmodic, antioxidant properties, anti-hyperlipidemic and anti-atherosclerotic, anticancer, anti-anxiety, antipyretic and wound healing^{43,47}. *Boerhaavia diffusa* rejuvenates the liver and male reproductive system, cleanses the kidneys, and helps to get rid of renal calculi. Roots are used as an

immunomodulator, hepatoprotective, antidiabetic, antibacterial, antistress, antiaging, anticancer, antimalarial, and anticonvulsant^{48,53}.

MATERIAL AND METHODS:

Plant Materials: In the present study, all the selected parts, such as the stem bark of *Ficus religiosa*, the stem of *Tinospora cordifolia*, and root of *Boerhaavia diffusa*, were collected from the surrounding areas of the Nagore district of Rajasthan in June. Leaves of *Moringa olifera* were collected from surrounding areas of Hyderabad. All the selected parts were authenticated by Dr. Md. Mustafa, Botanist, Department of Botany, Kakatiya University, Warangal, Telangana (Accession no. 1092, 1094, 1096, 1098).

Preparation of Plant Extracts: Dried parts of selected plants were powdered by using a mechanical grinder. The powders were passed through mesh sieve 44 and stored in airtight containers. The powdered sample was defatted with petroleum ether and kept for 72 hours at room temperature. 100 gm of each dried powdered sample was extracted with ethanol and distilled water (70:30) using the Soxhlet Apparatus for 24 hours. The solvent was removed to get the solid extract. The percentage yield of extracts for *Ficus religiosa* (4.75%), *Tinospora cordifolia* (5.67%), *Boerhaavia diffusa* (4.62%) and *Moringa oilfera* (7.54%).

Standardization of Selected Medicinal Plants:

Microscopy was performed to identify the microscopic characteristics of all selected plants in crude powdered form. Physicochemical parameters like ash value, loss on drying (moisture content), acid insoluble ash, water-soluble extractive and alcohol soluble extractive were carried out for all of the selected plants as per WHO guidelines⁵⁴. The hydroalcoholic extracts of all the selected plants were subjected to phytochemical screening to identify phytoconstituents like carbohydrates, proteins, alkaloids, tannins and phenolic compounds, saponins, and glycoside^{55,57}. Analysis of heavy metal as lead, arsenic, cadmium, mercury and chromium were analyzed in powder form of all the selected plants using Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES)^{58,59}.

Determination of *In-vitro* Antioxidant Activity:

In-vitro, the antioxidant activity of hydroalcoholic extracts of all selected plants was determined by DPPH free radical scavenging. A 0.25-millimolar methanolic solution of DPPH (2, 2-Diphenyl-2-picrylhydrazyl) was prepared. 1.0ml of methanolic DPPH solution was added to 1.0ml of hydroalcoholic extract in methanol, consisting of 0.01-0.05mg of the extract. The reaction mixture was shaken well and kept in the dark place at room temperature for 10 minutes. The absorbance of the mixture was measured at 517 nm using a double beam UV-Visible spectrophotometer. The same procedure was followed with ascorbic acid, which was used as a reference. All tests and reference standards were done in triplicates^{60,63}.

The average result obtained was used for the calculation of DPPH free radical scavenging ability (%) by using the formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{con}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{con}} \times 100}$$

Where, Abs control is the absorbance of DPPH radical +methanol. Abs sample is the absorbance of DPPH radical +extract/sample.

The antioxidant effects of each extract were expressed in terms of IC₅₀ (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the graph after plotting the percentage DPPH inhibition versus extract concentration.

RESULTS AND DISCUSSION: The present study focuses on quality control parameters such as powder microscopy, physicochemical parameters, and heavy metal testing. Preliminary phytochemical screening and *in-vitro* antioxidant studies were performed with the selected plants' hydroalcoholic extracts. Powder microscopy of stem bark of *Ficus religiosa* showed sclereid, stone cells, cork cells, fibre and latex cells **Fig. 1**. Stem *Tinospora cordifolia* showed tracheids, cork cells, phloem fibre, starch grains **Fig. 2**. Leaves of *Moringa olifera* showed epidermis cells with stomata, fibres, pitted vessels and starch grains **Fig. 3**. Roots of *Boerhaavia diffusa* cork cells showed pitted vessels and starch grains **Fig. 4**.

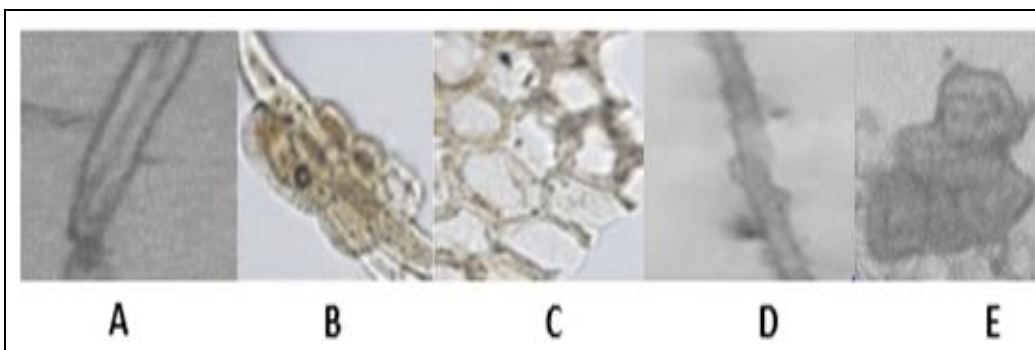


FIG. 1: MICROSCOPICAL CHARACTERS OF STEM BARK OF *FICUS RELIGIOSA*. A- SCLEREID, B- STONE CELLS, C- CORK CELLS, D-FIBRE, E-LATEX CELLS

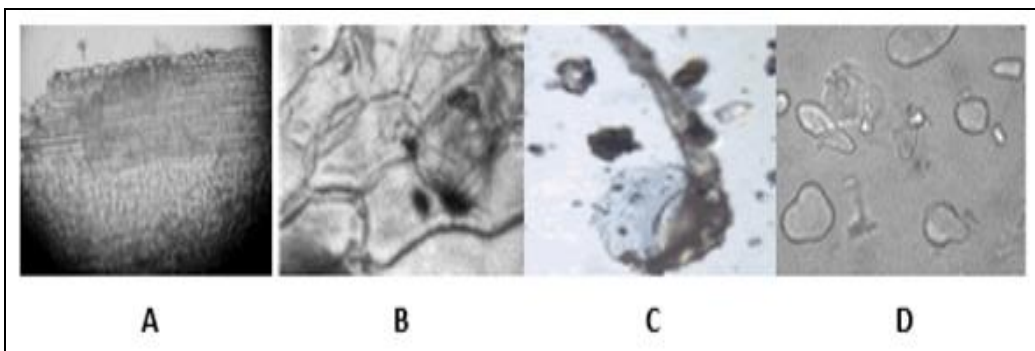


FIG. 2: MICROSCOPICAL CHARACTERS OF STEM OF *TINOSPORA CORDIFOLIA*. A- TRACHEIDS, B- CORK CELLS, C- PHLOEM FIBRE, D- STARCH GRAINS

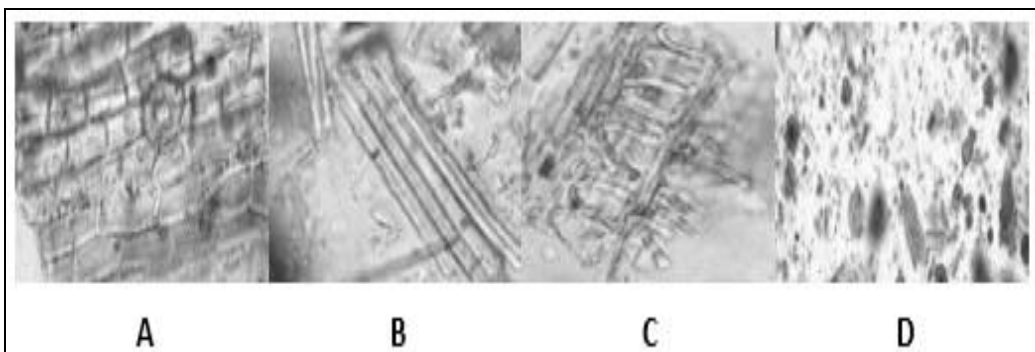


FIG. 3: MICROSCOPICAL CHARACTERS OF LEAVES OF *MORINGA OLIFERA*. A- EPIDERMIS CELLS WITH STOMATA, B- FIBRES, C- PITTED VESSELS, D- STARCH GRAINS

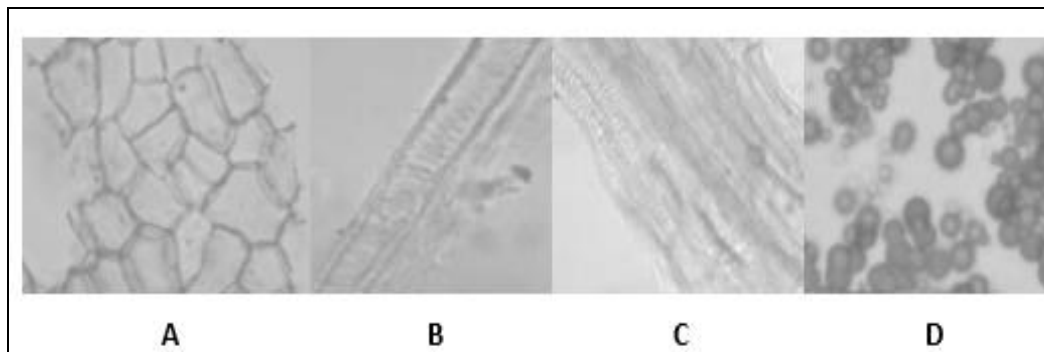


FIG. 4: MICROSCOPICAL CHARACTERS OF ROOT OF *BOERHAEVIA DIFFUSA*. A- CORK CELLS, B AND C- PITTED VESSELS, D-STARCH GRAINS

The results of various preliminary phytochemical screenings of hydroalcoholic extracts of selected plants are shown in **Table 1**. All the extracts

showed the presence of phytoconstituents like alkaloids, proteins, carbohydrates, tannins, phenols, saponins, glycosides and steroids.

TABLE 1: RESULTS OF PRELIMINARY PHYTOCHEMICAL SCREENING OF THE HYDROALCOHOLIC EXTRACT OF SELECTED PLANTS

S. no.	Plant Constituents (Test / Reagent)	<i>Ficus religiosa</i>	<i>Tinospora cordifolia</i>	<i>Moringa olifera</i>	<i>Boerhaavia diffusa</i>
Alkaloids					
1	Mayer's reagent	+	+	+	+
	Dragendroff's reagent	+	+	+	+
	Wagner's reagent	+	+	+	+
Glycosides					
2	Killer-Killani test	+	+	+	+
	Borntrager test	+	+	+	+
Carbohydrates					
3	Molisch's reagent	+	+	+	+
	Fehling solution	+	+	+	+
Sterols					
4	Salkowski test	+	+	+	+
Phenolic Compounds & Tannins					
5	Ferric chloride test	+	+	+	+
	Lead acetate solution	+	+	+	+
Proteins & Amino Acids					
6	Biuret test	+	+	+	+
	Ninhydrin Test	+	+	+	+
Flavanoids					
7	Ammonia test	+	+	+	+
Saponins					
8	Foam test	+	+	+	+
	Sodium bicarbonate test	+	+	+	+

The various physicochemical parameters of powders were performed as per the Ayurvedic Indian Pharmacopoeia of India, and the results are

given in **Table 2**. The values of all parameters for the selected medicinal plants were within the range of the standard limit⁶⁴.

TABLE 2: RESULTS OF PHYSICOCHEMICAL PARAMETERS OF SELECTED PLANTS

Physicochemical Parameter	% in <i>Ficus religiosa</i> Stem Bark Powder	% in <i>Tinospora cordifolia</i> Stem Powder	% in <i>Moringa olifera</i> Leaves powder	% in <i>Boerhaavia diffusa</i> Root Powder
Loss on drying	3.94%	2.65%	2.8%	2.06%
Total Ash	6.30%	11.22%	4.04%	10.9%
Acid insoluble ash	0.24%	2.16%	2.4%	1.68%
Water-soluble extractive	8.24%	9.02%	7.63%	11.96%
Alcohol soluble extractive	6.8%	2.98%	9.34%	2.46%

Heavy metal testing was done on the crude powdered form of all the selected parts of different plants by using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and the results are given in **Table 3**. Except for chromium, the concentration of all the tested heavy metals was below 1 ppm per kg of crude powdered drug.

The concentration of it was 1.82 ppm in the stem bark of *Ficus religiosa*, 2.96 ppm in the stem of *Tinospora cordifolia*, 1.42 in the leaves of *Moringa olifera* and 2.24 in the root of *Boerhaavia diffusa*. The maximum concentration of chromium was present in the powder of the stem of *Tinospora cordifolia*.

TABLE 3: RESULTS OF HEAVY METAL ANALYSIS OF SELECTED PLANTS

S. no.	Parameters	Results (ppm/ Kg)			
		<i>Ficus religiosa</i>	<i>Tinospora cordifolia</i>	<i>Moringa olifera</i>	<i>Boerhaavia diffusa</i>
1.	Lead as Pb	<1.00	<1.00	<1.00	<1.00
2.	Arsenic as As	<1.00	<1.00	<1.00	<1.00
3.	Cadmium as Cd	<1.00	<1.00	<1.00	<1.00
4.	Mercury as Hg	<1.00	<1.00	<1.00	<1.00
5.	Chromium as Cr	1.82	2.96	1.42	2.24

The percentage of DPPH inhibition of hydroalcoholic extract of all the selected plants was compared with ascorbic acid as the standard is given in **Table 4** and **Fig. 5**. All the hydroalcoholic extracts showed lesser DPPH free radical scavenging effect than ascorbic acid. The maximum antioxidant effect was shown by the hydroalcoholic extract of the stem of *Tinospora cordifolia*. DPPH free radical scavenging activity

has been considered due to the redox reaction of the extract's phytoconstituents, which helps neutralize free radicals. The IC₅₀ value (micromolar concentration required to inhibit DPPH radical formation by 50%) is the minimum for ascorbic acid (standard). In **Fig. 6**, IC₅₀ value for the extracts of the selected plants is given, and the value is minimum for the hydroalcoholic extract of the stem of *Tinospora cordifolia*.

TABLE 4: % DPPH INHIBITION OF HYDROALCOHOLIC EXTRACT OF SELECTED PLANTS AND ASCORBIC ACID

Concentration (mcg/ml)	% DPPH Inhibition of Different of Hydroalcoholic Extract (mean±S.D.)				
	<i>Ficus religiosa</i>	<i>Tinospora cordifolia</i>	<i>Moringa olifera</i>	<i>Boerhaavia diffusa</i>	Ascorbic acid
10	26.84 ± 0.001	44.58 ± 0.001	31.53 ± 0.003	38.35 ± 0.002	54.62 ± 0.001
20	39.89 ± 0.001	56.56 ± 0.002	37.35 ± 0.002	44.58 ± 0.001	68.07 ± 0.001
30	48.93 ± 0.001	58.77 ± 0.003	42.77 ± 0.002	51.00 ± 0.001	83.33 ± 0.001
40	57.01 ± 0.001	62.78 ± 0.001	50.40 ± 0.002	55.62 ± 0.002	87.55 ± 0.001
50	62.12 ± 0.002	68.87 ± 0.001	56.43 ± 0.001	59.24 ± 0.002	91.97 ± 0.001

Mean= Average of three absorbances, S.D. =Standard deviation.

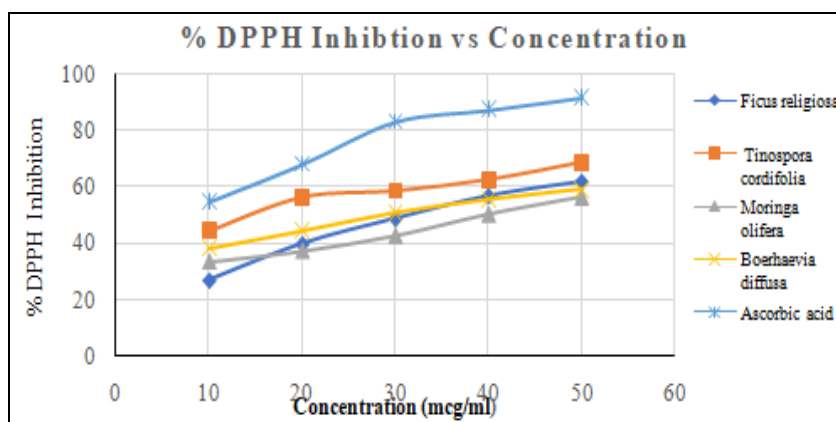


FIG. 5: COMPARATIVE DPPH % INHIBITION OF HYDROALCOHOLIC EXTRACTS OF SELECTED PLANTS AND ASCORBIC ACID

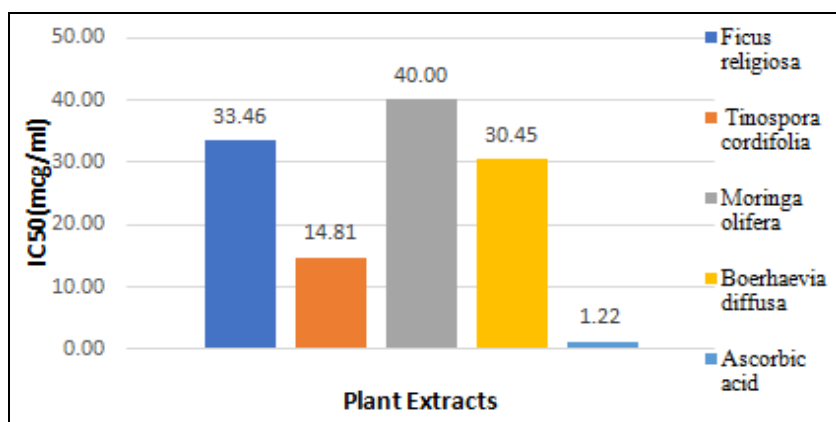


FIG. 6: IC 50 VALUES OF ASCORBIC ACID OTHER PLANT EXTRACTS

Standardization ensures that every medicine or herbal product on the market contains the same constituent and has the same pharmacological effect. Powder microscopy, physicochemical

parameters, phytochemical screening, and heavy metal testing were used to investigate the quality control parameters of the selected medicinal plant materials.

This scientific validation is useful for assessing quality, adulteration, purity, and uniformity in the phytoconstituents of the selected medicinal plants. All the selected plants showed good DPPH free radical scavenging activity.

They could be used to develop an herbal formulation for treating diseases associated with a high level of free radicals. The IC₅₀ value is the minimum for the hydroalcoholic extract of the stem of *Tinospora cordifolia*, but it is more than IC₅₀ value of ascorbic acid. The potential pharmacological utility of all these selected medicinal plant parts would be benefited from a further study on these standardized medicinal plants.

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