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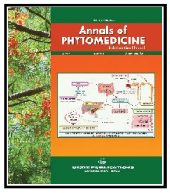

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Comparative phytochemical investigations and nitric oxide free radical scavenging activity of selected medicinal plants

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Abstract

Globally, several medicinal plants used for treatment of various diseases. In the present, study few medicinal plants have been selected such as stem bark of *Ficus religiosa* L., stem of *Tinospora cordifolia* Miers, leaves of *Moringa oleifera* Lamk and roots of *Boerhavia diffusa* L. and all these plant materials are subjected for qualitative phytochemical investigations, quantitative phytochemical investigations, and nitrous oxide free radical scavenging activity. The powdered plant materials of selected plants were extracted with ethanol and distilled water mixture (70:30) by using Soxhlet apparatus; all the hydroalcoholic extracts showed presence of phytochemicals like glycosides, alkaloids, carbohydrates, proteins, amino acids, tannins, phenols, phytosterols and saponins. Total phenolic, total alkaloid, and total flavonoid contents were estimated for quantitative phytochemical investigations. Highest quantity of total alkaloid was found in hydroalcoholic extract of roots of *B. diffusa* (4.24 ± 0.002). Maximum amount of total phenolics content (2.16 ± 0.03) and total flavonoid content (9.91 ± 0.021) were found in hydroalcoholic extract of *T. cordifolia* stem.

Nitric oxide free radical scavenging assay was performed for all hydroalcoholic extracts and compared with reference catechin. Maximum % NO inhibition was found with reference standard catechin (96.3 ± 0.005), followed by the hydroalcoholic extract of *T. cordifolia* (75.8 ± 0.004). IC_{50} value was found minimum for catechin (13.11 µg/ml) and in all extracts *T. cordifolia* hydroalcoholic extract has minimum IC_{50} value (45.05 µg/ml).

1. Introduction

Many medicinal agents are obtained from natural sources from ancient era. A huge number of medicinal plants are used for maintenance of health and treatment of various diseases worldwide (Devi *et al*., 2023). All these medicinal plants were used in various traditional system of medicine of India from thousands of years (Nair *et al*., 2005). India is known as botanical garden of entire globe due to the biggest producer of useful medicinal plants (Mohanasundari *et al*., 2007; Cragg and Newmann, 2001). In India, around 6000 are being used by people as traditional medicine, folk medicine, and herbal medicine, but out of this, only 3000 plants are officially established for their medicinal use (Rajshekharan, 2002). The medicinal properties of these medicinal herbs may be due to the phytochemicals present in it. These phytochemicals are categorized in primary metabolites (sugars, amino acids, colored pigments, *etc.*) and secondary metabolites (triterpenoids, volatile oil, alkaloids, flavonoids, saponins, *etc.*). These phytochemicals merged with the defense system by binding with the nutrients and help in fighting against diseased and stressed conditions (Krishnaiah *et al*., 2007). Increased utilization of these medicinal plants as alternative remedies, pharmaceutical agents, natural products in cosmetic and perfumes, moved the interest of researchers in the exploitation of these medicinal plants and provided huge opportunities (Kirtikar and Basu, 1993). In the present study, four Indian medicinal plants have been selected for estimation of different phytochemicals and all these plants have huge medicinal importance in different traditional system and folk medicine (Chopra and Chopra, 1958; Sivarajan and Balachandran, 1999). The selected plants and parts are the stem bark of *F. religiosa*, stem of *T. cordifolia*, leaves of *M. oleifera* and roots of *B. diffusa*.

The aim of present study is to do the comparative phytochemical investigations of hydroalcoholic extracts of selected plant parts of the selected Indian medicinal plants and estimation of nitric oxide free radical scavenging activity.

1.1 Botanical description and phytoconstituents of selected plants

Ficus religiosa L. (*F. religiosa*) is belonging to family-Moraceae, is a popular as bodhi tree and has immense attention in Indian heritage due to its sacred, mythological, and medicinal importance (Prasad *et al*., 2006; Chandrasekar *et al*., 2010). This plant is an ever-blooming tree, 20-35 meters in height and 1.5-2 meters wide. The outer bark is grey, or ash coloured with 2.0-2.5 cm thick sloughed smooth irregular brownish scales. Middle bark sections are reddish to brownish. Light yellowish or orangish-brown granular tissue is found in layers of the inner part of the bark. The bark's taste is astringent and odourless (Ayurvedic Pharmacopoeia, 1999; Koilpillai *et al.,* 2010). The bark consists of phytosterols like lanosterol, β-sitosterol and its glucoside (β-sitosteryl-d-glucoside) and stigmasterol. The bark of *F. religiosa*

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comprises around 8.7% of total tannin content and other phytoconstituents are vitamin K1, n-octacosanol, methyl oleonate and lupen-3-one. Bergapten and bergaptol are substituted furanocoumarins (Sharma *et al*., 2019).

Tinospora cordifolia Miers. (*T. cordifolia*) is belonging to family-Menispermaceae. Its common vernacular names are amrita, guduchi, shindilkodi, giloy, *etc.* (Sinha *et al*., 2004; Sharma and Singh, 2010). It's stem is luscious, filiform, long, fleshy, and with aerial roots which are coming out from stem and climbs downward. Stem is twisted longitudinally, and colour of the stem bark is pale milky to greyish. The chemical constituents of stem belong to different classes like alkaloids, glycosides, steroids, phenolics, aliphatic compounds, polysaccharide. The phytoconstituents isolated from the stem are: cordifolioside A and B, berberine, 1,2-Substituted pyrrolidine, amritoside A, B, C and D, octacosanol (Sharma *et al*., 2019; Garg and Garg, 2018).

Moringa oleifera Lamk (*M. oleifera*) is belonging to family-Moringaceae. It is popularly called 'Miracle Tree' (Dhongade *et al*., 2017; Fahey, 2017). It is a fast-growing, medium-sized, evergreen tree approximately 10-12 m long and widely grown throughout India. Leaves are tri-pinnate and usually flowering occurs after six months of planting (Rani *et al*., 2018). It contains phytoconstituents like kaempferitrin, isoquercetin, rhamnetin, kaempferol and quercetin, zeatin, ascorbic acid, phenolic, flavonoids, vitamin E (Fidrianny *et al*., 2021).

Boerhavia diffusa L. (*B. diffusa*) is belonging to family-Nyctaginaceae. It is known as Punarnava in Sanskrit which means that rejuvenates the old body (Nayak and Thirunavoukkarasu, 2016). *B. diffusa* is a widely spreading diffusely branched pubescent, prostrate and glabrous herb. The root is well developed, fusiform and is about 30-50 cm deep in soil. Diameter is 0.2 to 1.5 cm, yellowish to brownish grey in colour, outer surface is soft but sometimes uneven due to the presence of minute long striations. The plant has superimposed biaxial construction, and the young shoots are born on the axis's upper surface. It contains boeravinones A-1, B-1, C-2, D, E and F, behenic acid, beta-sitosterol, boerhaavic acid, borhavine, borhavone, campesterol, daucosterol, beta-ecdysone, hentriacontane N, hypoxanthine-9-l-arabinofuranoside, ursolic acid, and 5,7-dihydroxy-3,4-dimethyoxy-6,8-dimethyl flavone (Patil and Bhalsing, 2016).

1.2 Traditional uses of selected plants

The bark of *F. religiosa* is used in treatment of gonorrhoea and other infections like bacterial, viral, protozoal infections, neurodegeneration, ulcer, hepatic disorders, kidney disorders, hepatic disorders (Singh *et al*., 2011). It is also used as antiulcer astringent, antidiarrhoeal, antineoplastic, nephroprotective (Gregory *et al*., 2016). The extract of stem bark was also found to have antiseptic and astringent properties (Goyal, 2014).

In the ayurvedic medicine system, *T. cordifolia* stem is used for the treatment of jaundice muscle contraction, pyrexia, neoplasm, urinary diseases, viral hepatitis, hyperlipidaemia, digestive disturbances diabetes, immunity disorders and liver diseases (Devprakash *et al*., 2011; Upadhyay *et al*., 2010).

Leaves of *M. oleifera* are used for treatment of bacterial and fungal infection, liver diseases (Sharma and Singh, 2010), inflammation, depression, cancer, pain, neurologic disorders, microbial disorders in the traditional medicine system of South Asia (Mahajan *et al*., 2007).

Roots of *B. diffusa* is used as bitter tonic, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiotonic, and laxative (Patil and Bhalsing, 2016). It is also used in the treatment of inflammation, cough, bronchitis and general debility, leucorrhoea, ophthalmia, jaundice, anaemia, dyspepsia, constipation (Manu and Kuttan, 2009; Singh and Udupa, 1972a).

2. Materials and Methods

2.1 Plant materials

In the present study, all the selected parts, such as stem bark of *F. religiosa*, stem of *T. cordifolia* and root of *B. diffusa* were collected from the surrounding areas of the Nagore district of Rajasthan in June. Leaves of *M. oleifera* 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. The Herbarium Accession no. of selected plants: *T. cordifolia* (1092), *M. oleifera* (1094), *B. diffusa* (1096) and *F. religiosa* (1098).

2.2 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 h, at room temperature (Tahkur *et al*., 2016; Tiwari *et al*., 2011). 100 g of each dried powdered sample was extracted with a mixture of ethanol and distilled water (70:30) by using the Soxhlet apparatus for 24 h. The solvent was removed to get the solid extract. Percentage yield of extracts for *F. religiosa* (4.75%), *T. cordifolia* (5.67%), *B. diffusa* (4.62%) and *M. oleifera* (7.54%).

2.3 Qualitative phytochemical investigation

Hydroalcoholic extract of all selected parts of all Indian medicinal plants were analysed for qualitative phytochemical analysis for identification of the different phytoconstituents (Khandelwal, 2000; Mishra and Khushtar, 2021).

2.3.1 Estimation of alkaloids

In individual test tubes, the hydroalcoholic extracts were taken and dissolved in dilute HCl and then filtered. Resulted filtrates were used for determination of alkaloids.

- **Wagner's reagent test:** To all the filtrates in separate test tubes, 0.5 ml of Wagner's reagent was added. By producing a reddish-brown precipitate, alkaloid presence was verified.
- **Mayer's reagent test:** Add 0.5 ml of Mayer's reagent to 2-3 ml of all the filtrates. Alkaloids are identified when a green or white precipitate forms.
- **Dragendorff's reagent test:** Dragendorff's reagent was diluted to 0.5 ml and added to 2-3 ml of each filtrate. Precipitate with an orange-brown colour formed, indicating the presence of alkaloids.

2.3.2 Estimation of glycosides

- **Killer-Killiani test:** 3 ml of hydroalcoholic extract was mixed with 2 ml of glacial CH_3COOH containing of 0.2 ml of 5% $FeCl_3$ was added. Then in resulting solution one ml of conc. H_2SO_4 was mixed. A red to brown colour appears at the juncture point of

two liquids due to these two layers were appeared. Top layer became blue to green, and this confirms the occurrence of cardiac glycosides.

- **Borntrager's test:** Each extract was taken around 3 ml, to this 2 ml of dil. H_2SO_4 was mixed. The contents were boiled and filtered. The cooled filtrate was treated with 2 equal volumes of dichloromethane (CH_2Cl_2) or chloroform ($CHCl_3$) and shaken well. Ammonia was added to the lower organic layer. This ammoniacal layer turned rose pink to red, which confirms the presence of Anthraquinone glycoside.

2.3.3 Estimation of carbohydrates

In individual test tubes, the hydroalcoholic extracts were taken, dissolved in water, and filtered. This was used for test of alkaloids.

- **Molisch test:** 2 ml of filtrate of plant extract was put separately in a test tube and 1 ml of Molisch's reagent was added. Around 2 ml concentrated from side of test tube, sulphuric acid was added. A purple or reddish colour ring is formed at the meeting point of two liquids, which indicates occurrence of carbohydrates.
- **Fehling test:** 1ml of each Fehling's solution A and B was mixed in a test tube, to this add 2 ml of the filtrate, then test tubes were heated for 10 min. The deep blue colour changed to reddish brown, which shows the presence of carbohydrates (reducing sugars)

2.3.4 Estimation of phytosterols

- **Salkowski's test:** Each plant's extract was combined with around two ml of $CHCl_3$ and add two ml of conc. H_2SO_4. The emergence of yellow fluorescence confirmed the presence of the phytosterols.

2.3.5 Estimation of phenolic compounds and tannins

- **$FeCl_3$ test:** Each extract's (2 ml) aqueous extract solution was combined with 0.5 ml of 5% $FeCl_3$. The appearance of a deep blue colour confirmed phenolic compounds.
- **Lead acetate test:** Aqueous solution of each extract (2 ml) was combined with 0.5 ml of lead acetate solution (1%) and the emergence of a white precipitate confirmed occurrence of tannins.

2.3.6 Estimation of proteins and amino acids

- **Biuret test:** To the extract, 0.5 ml of 4% NaOH solution was added; followed by addition of $CuSO_4$ solution (1%). Appearance of pink colour confirms the occurrence of protein.
- **Ninhydrin test:** To 2 drops of ninhydrin solution was combined to the extract solution and then, it was heated on a boiling water bath for 10 min. The development of a purplish colour confirms occurrence of proteins, peptides, or amino acids.

2.3.7 Estimation of flavonoids

- **Shinoda test:** To the extract, 5 ml of absolute ethanol, and 0.5 ml of concentrated HCl was added. Then put a pinch of magnesium turnings. The appearance of pink colour shows the occurrence of flavonoids.
- **Reagent test:** 0.5 ml of NaOH solution was mixed to the extract solution. A sharp yellow colour appears, that gets decolorised by addition of dilute acid, which confirms the occurrence of flavonoids.

2.3.8 Estimation of saponins

- **Foam test:** Specified amount of extract was shaken with 2-3 ml of distilled water. The development of persistent foam confirms the occurrence of saponins.

2.4 Quantitative phytochemical investigations

2.4.1 Estimation of alkaloids

The total alkaloid is estimated by taking 5 g of powdered plant material into a separate beaker mixed with 20% ethanolic acetic acid (200 ml) and cover with lid. This was kept aside for 4 h at ambient temperature. Content was filtered and heated on a water bath till the amount was reduced to one-fourth. Then, this solution was precipitated by adding concentrated ammonium hydroxide in dropwise manner till complete precipitation. Then, filtration was done to collect precipitate then it was air dried, and weight was taken. Percentage of total alkaloid was estimated in milligram per gram of sample (Harborne, 1984; Kokate and Gokhale, 2008)

Total alkaloid content (%)= 'w' of residue x 100/ wt. of sample

2.4.2 Estimation of total phenolic contents in the plant extracts

The spectrophotometric procedure is used for calculation of the total phenolic concentration in extracts of selected plant parts (Pawaskar and Ranade, 2022; Krishnarao and Raja Rajeswari, 2023). 100 milligram of GAE was solubilized in methanol (10 ml) and then diluted till 100 ml with methanol in volumetric flask, which gives 1mg/ml stock solution of gallic acid for further dilutions. To 0.5 ml of resulting solution of each extract, 0.5 ml diluted Folins- Ciocalteu's solution was added and 2.5 ml 20% Na_2CO_3; volume was adjusted to 10 ml with distilled water. For development of colour, all test samples were incubated for 40 min at ambient temperature. Absorbance was estimated with the help of double beam UV spectrophotometer at 760 nm (λ_{max}) by using a blank. A standard graph for gallic acid (GAE) was prepared in concentration (20-100 mcg/ml). The samples and standard solutions were prepared in triplicate for the estimation. The total phenolics content in each hydroalcoholic extract was estimated in terms of gallic acid equivalent (mg GAE/g extract) by using calibration graph.

2.4.3 Estimation of total flavonoid in the plant extracts

Estimation of total flavonoid was done by aluminium chloride complex forming assay and analysis was done by colorimetric method (Eshbakova *et al*., 2022). 100 milligram of quercetin was solubilized in methanol (10 ml) and then diluted up to 100 ml with methanol which gives 1mg/ml stock solution of quercetin. Quercetin was used as reference for estimation of total flavonoid concentration. To 1.0 ml of each plant part extract, 3 ml of methanol was mixed in a 10 ml of flask. 0.2 ml of aluminium chloride (10 %) was mixed to it then put sodium acetate (0.2 ml). Then, it was diluted till 10 ml by adding distilled water. All the test samples were kept aside for 30 min at the temperature 40ºC for development of colour. A calibration curve of quercetin (QE) was prepared in the same manner in the concentration (20-100 mcg/ml). The absorbance was measured for test and standard solutions using a reagent blank at 430 nm wavelength by UV-visible spectrophotometer. The values were expressed in terms of quercetin equivalent (QE) mg/g extract.

2.5 Nitric oxide scavenging activity

Nitric oxide assay is used for estimation of *in vitro* the antioxidant ability of all selected plant extracts. Sodium nitroprusside solution was spontaneously decomposed at biological pH and generates radicals of nitric oxide (Marcocci *et al*., 2007; Alam *et al*., 2013). It reacts with oxygen and gives oxide ions of nitrogen (nitrate and nitrite). Estimation of these ions is done by Griess reagent. Three ml of sodium nitroprusside was solubilized in phosphate buffer saline (10 millimole/litre of pH 7.4), and it was mixed to various concentrations of extracts (0.20-0.10 mg/ml) and it was allowed to stand at 25°C for 2-2.5 h. To this, 0.5 ml of Griess reagent was mixed. The absorbance was taken by a UV- spectrophotometer at 546 nm. In similar way, absorbance was measured for reference standard catechin. Absorbance for all tests samples and reference standard were analysed in triplicates. The mean value was used for estimation of % NO inhibition by using formula:

Nitric oxide scavenging activity (%) $=Abs_{con} - Abs_{sample} / Abs_{con} \times 100$

where: Abs_{con} is the absorbance of NO radical + methanol.

Abs_{sample} is the absorbance of NO radical + extract/sample.

IC_{50} is termed as the micromolar concentration needed for 50% inhibition of NO radical formation was calculated from the graph.

3. Results

In this study, different hydroalcoholic extract of selected plants has been evaluated for preliminary phytochemical screening, total phenolic content, flavonoids, and tannins.

3.1 Qualitative phytochemical investigation

Results of qualitative phytochemical investigation of all the hydroalcoholic extracts of selected plant materials showed the occurrence of phytochemicals such as alkaloids, proteins, phenolic compounds, carbohydrates, flavonoids, tannins, saponins, phytosterols and glycosides.

3.2 Quantitative phytochemical analysis

Quantitative phytochemical investigation was done by estimation of total alkaloid (gravimetric method), total phenolic content and total flavonoid content for hydroalcoholic extract of all selected medicinal plants. Standard calibration curve for gallic acid is given in Figure 1and for quercetin is given in Figure 2.

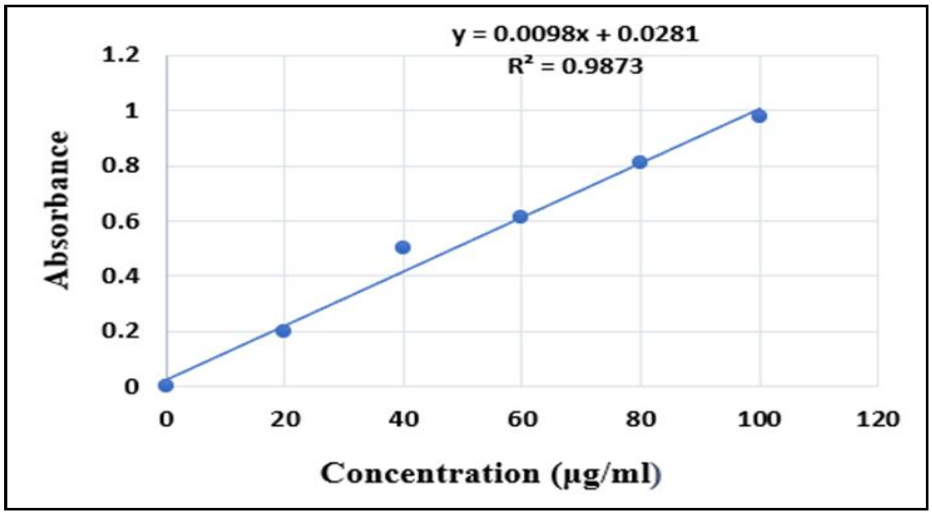

Figure 1: Standard calibration curve of gallic acid for total phenolic content.

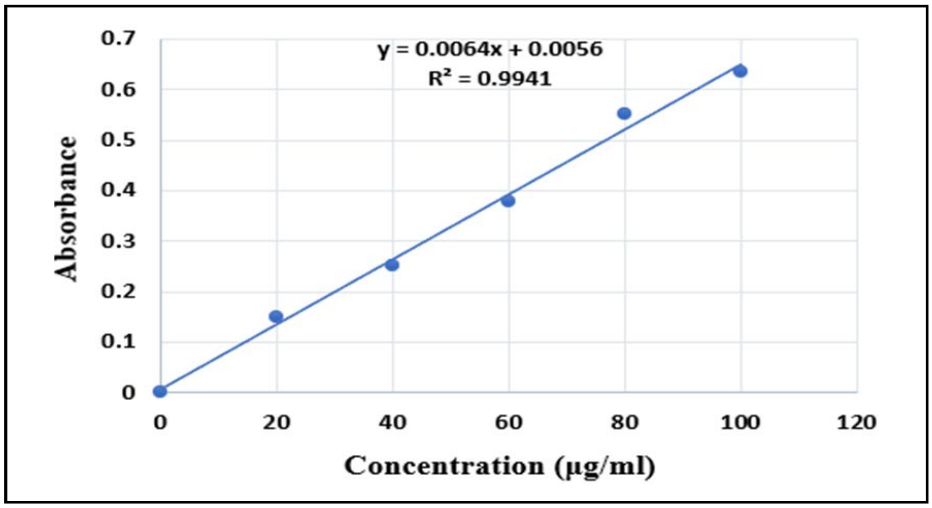

Figure 2: Standard calibration curve of quercetin for total flavonoid content.

These calibration curves were used for estimation of total phenolic content and flavonoid content. Results of quantitative phytochemical investigation, for all extracts of chosen plant materials are given in Table 1 and comparative study is given in Figure 3.

Table 1: Results of quantitative phytochemical investigation of plant extracts

Plant extracts	Total alkaloid *(%w/w)	Total phenolics concentration *(mg GAE/g)	Total flavonoids concentration *(mg QE/g)
F. religiosa	1.49 ± 0.02	1.55 ± 0.002	3.04 ± 0.003
T. cordifolia	2.86 ± 0.24	2.16 ± 0.03	9.91 ± 0.021
M. oleifera	2.06 ± 0.01	1.35 ± 0.04	2.41 ± 0.001
B. diffusa	4.24 ± 0.002	1.75 ± 0.02	3.35 ± 0.012

*Values are represented as the mean of triplicate ± SD.

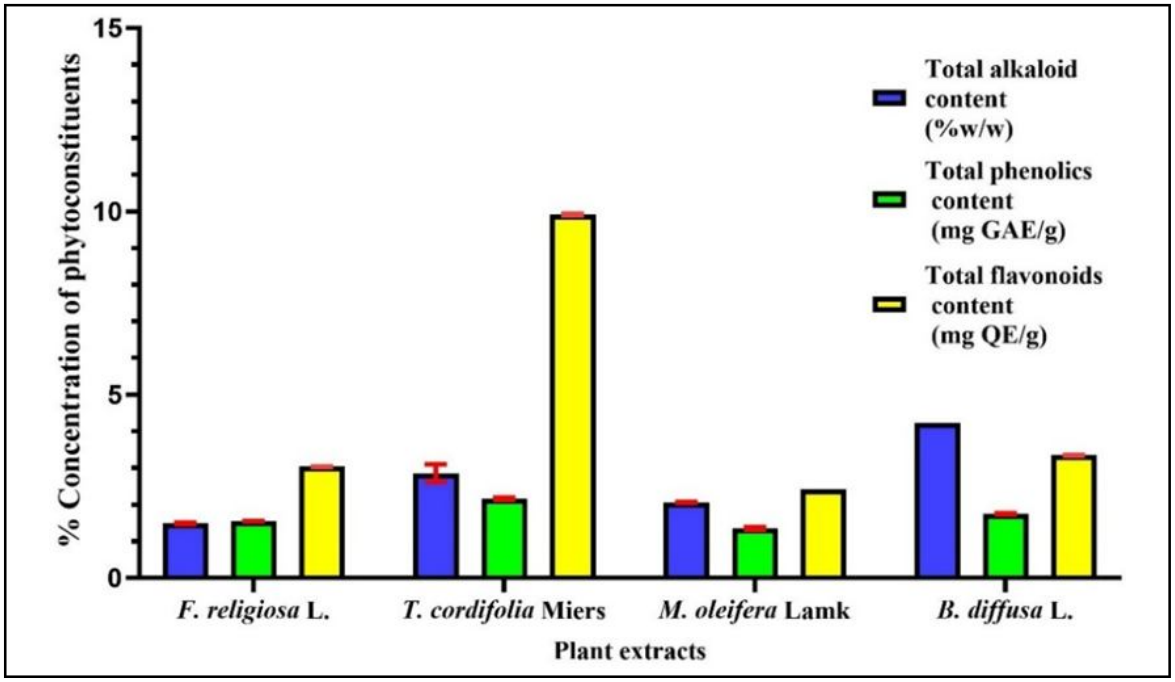

Figure 3: Comparative quantitative phytochemical investigation for hydroalcoholic extracts.

3.3 Nitric oxide scavenging activity

Findings of nitric oxide assay for estimation of antioxidant ability are given in Table 2 for all hydroalcoholic extracts and standard catechin and comparative study % nitric oxide inhibition is given in Figure 4. Results showed that maximum % nitric oxide inhibition was found with standard catechin (96.3 ± 0.005) and hydroalcoholic extract of *T. cordifolia* is having (75.8 ± 0.004), which is as per standards. The % NO inhibition was found for *F. religiosa* (55.1 ± 0.005), *B. diffusa* (73.2 ± 0.003), and *M. oleifera* (65.1 ± 0.002).

Table 2: % NO inhibition for catechin and selected medicinal plant extracts

Conc. (µg/ml)	% NO inhibition of hydroalcoholic extracts* (mean ± Std. Dev.)				
	F. religiosa	*T. cordifolia*	*M. oleifera*	*B. diffusa*	Catechin
20	15.1 ± 0.002	36.4 ± 0.004	30.0 ± 0.007	23.5 ± 0.001	48.0 ± 0.003
40	28.0 ± 0.004	48.3 ± 0.007	36.6 ± 0.003	36.6 ± 0.003	68.3 ± 0.005
60	39.0 ± 0.004	60.1 ± 0.007	50.7 ± 0.001	49.0 ± 0.002	86.1 ± 0.001
80	49.2 ± 0.006	65.0 ± 0.003	59.4 ± 0.002	55.5 ± 0.001	89.6 ± 0.001
100	55.1 ± 0.005	75.8 ± 0.004	65.1 ± 0.002	73.2 ± 0.003	96.3 ± 0.005

* Values are represented as the mean of triplicate ± Std. Dev.

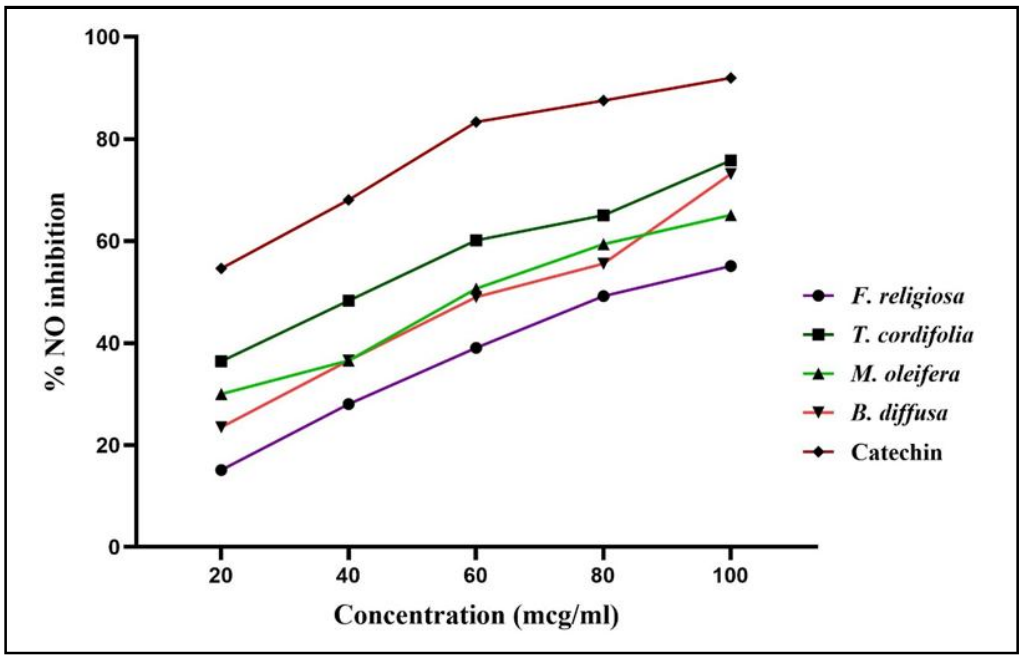

Figure 4: Comparative percentage NO inhibition for selected plants extracts and catechin.

IC_{50} is determined from graph for hydroalcoholic extracts of selected plant material and catechin is given in Figure 5. The IC_{50} value is found minimum for standard catechin (13.11 µg/ml) and in all extracts, the value is least for extract of *T. cordifolia* stem (45.05 µg/ml).

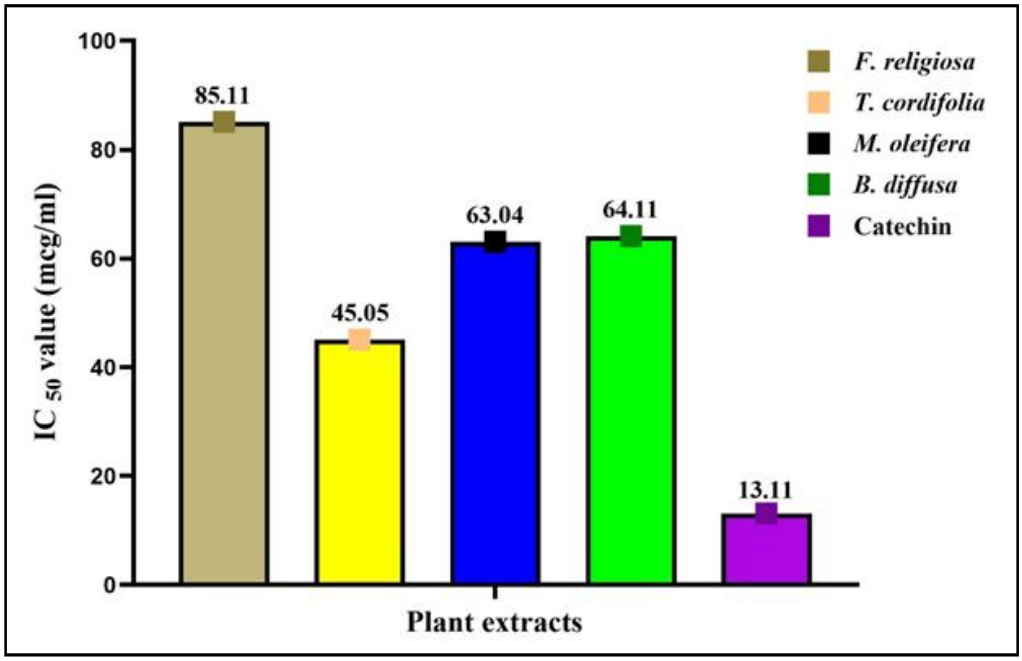

Figure 5: Comparative IC_{50} values of extracts of selected plants and catechin.

4. Discussions

Medicinal plants possess significant role in the treatment and management of different acute and chronic disease conditions and thus provide an inherent role in healthcare system (Venkatachalam *et al*., 2021). The % yield of hydroalcoholic extracts was found maximum for leaves of *M. oleifera* (7.54%), followed by the stem of *T. cordifolia* (5.67%), then the stem bark of *F. religiosa* (4.75%), and then roots of *B. diffusa* (4.62%). The results of the comparative qualitative phytochemical investigation of all selected parts of selected medicinal plants highlighted that the hydroalcoholic extracts of all selected plant parts were rich in the phytoconstituents such as such as alkaloids, proteins, phenolic compounds, carbohydrates, flavonoids, tannins, saponins, phytosterols and glycosides.

Findings of quantitative phytochemical investigation highlighted that showed that maximum quantity of total alkaloid was found in hydroalcoholic extract of roots of *B. diffusa* (4.24 ± 0.002), followed by hydroalcoholic extract of stem of *T. cordifolia* (2.86 ± 0.24), then hydroalcoholic extract of *M. oleifera* (2.06 ± 0.01), and hydroalcoholic extract of *F. religiosa* (1.49 ± 0.02). Total phenolics was found in hydroalcoholic extract of *T. cordifolia* stem (2.16 ± 0.03), followed by hydroalcoholic extract of *B. diffusa* (1.75 ± 0.02), then hydroalcoholic extract of *F. religiosa* (1.55 ± 0.002), and hydroalcoholic extract of *M. oleifera* (1.35 ± 0.04). Results of total flavonoid content showed that maximum amount of total flavonoid content was present in hydroalcoholic extract of *T. cordifolia* stem (9.91 ± 0.021), followed by hydroalcoholic extract of roots of *B. diffusa* (3.35 ± 0.012), then hydroalcoholic extract of *F. religiosa* (3.04 ± 0.003), and then hydroalcoholic extract of *M. oleifera* (2.41 ± 0.001).

Nitric oxide assay for scavenging of free radicals was performed in concentration range of 20 -100 µg/ml for all hydroalcoholic extracts and compared with reference catechin at the same concentration (Marcocci, *et al*., 1994). Maximum % NO inhibition was found with reference standard catechin (96.3 ± 0.005), followed by the hydroalcoholic extract of *T. cordifolia* (75.8 ± 0.004) and % NO inhibition for other hydroalcoholic extracts was *B. diffusa* (73.2 ± 0.003), *M. oleifera* (65.1 ± 0.002) and *F. religiosa* (55.1 ± 0.005). IC_{50} value for each plant hydroalcoholic extract and catechin was determined and the IC_{50} value was found minimum for catechin (13.11 µg/ml) and in all extracts *T. cordifolia* hydroalcoholic extract has minimum IC_{50} value (45.05 µg/ml) whereas, the extracts of *B. diffusa* (64.11 µg/ml) and *M. oleifera* (63.04 µg/ml) had almost similar IC_{50} value and maximum IC_{50} value was found for *F. religiosa* (85.11 µg/ml).

5. Conclusion

Hydroalcoholic extracts of selected plant parts of selected medicinal plants were found rich in phytoconstituents by qualitative phytochemical investigation such as glycosides, alkaloids, carbohydrates, proteins, amino acids, tannins, phenols, phytosterols and saponins. Quantitative phytochemical investigation showed that hydroalcoholic extract of stem of *T. cordifolia* had maximum amount total phenolics and flavonoids, whereas maximum amount of alkaloid was found in roots of *B. diffusa* than other three hydroalcoholic extracts. Nitric oxide free radical scavenging assay showed that maximum % NO inhibition was found in reference standard catechin, followed by the hydroalcoholic extract of *T. cordifolia*. IC_{50} value was found minimum for catechin and in all extracts. *T. cordifolia* hydroalcoholic extract has minimum IC_{50} value. Traditional importance of all the selected medicinal plants may be due to presence of these phytoconstituents. Based on the present investigation, it may be concluded that antioxidant nature these phytochemicals may be responsible for immense importance of these medicinal plants in pharmacy and phytotherapy. Polyherbal formulations can be developed by using these plants which can be used for the treatment of diseases due generation of high levels of free radicals.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Alam, M.N.; Bristi, N.J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm. J., **21**(2):143-152.

Ayurvedic Pharmacopoeia of India. (1999). Part-I, Volume-IV, published by Department of AYUSH, Ministry of Human and Family Welfare, Government of India.

Chandrasekar, S.B.; Bhanumathy, M.; Pawar, A.T. and Somasundaram, T. (2010). Phytopharmacology of *Ficus religiosa*. Pharmacognosy Rev., **4**(8):195-199.

Chopra, R.N. and Chopra, S. (1958). Indigenous drugs of India. Dhur and Sons: Calcutta, second edition., pp: 606.

Cragg, G.M. and Newman, D.J. (2001). Natural product drug discovery in the next millennium. Pharm. Biol., **39**(1):8-17.

Devi, D.; Singh, S.; Moond, M.; Matoria, P.; Kumari, S.; Saini, K. and Sharma, R.K. (2023). Phytochemical analysis and antioxidant potential of (L.) seeds. Ann. Phytomedicine., **12**(1):502-507. Doi: http://dx.doi.org/10.54085/ap.2023.12.1.7

Devprakash, S.K.; Subburaju, T.; Gurav, S. and Singh, S. (2011). *Tinospora cordifolia*: A review on its ethnobotany, phytochemical and pharmacological profile. Asian J. Biomed. Pharm. Sci., **4**(1):291-302.

Dhongade, H.K.J.; Paikra, B. and Gidwani, B. (2017). Phytochemistry and pharmacology of *Moringa oleifera* Lamk. J. Pharmacopuncture., **20**(3):194-200.

Eshbakova, U.K.; Ashirmatova, N.; Mamarasulov, B.; Khasanova, K.; Komilov, B. and Davranov, K. (2022). Total phenol and flavonoid content, antibacterial and antioxidant activity of extract and fractions of medicinal plants of the Rumex (Polygonaceae) family in the flora of Uzbekistan. Ann. Phytomedicine., **11**(2):463-472.

Fahey, J.W. (2017). *Moringa oleifera*: A review of the medicinal potential. Acta Hortic., (1158):209-224. Doi:10.17660/ActaHortic. 2017. 1158.25

Fidrianny, I.; Kanapa, I. and Singgih, M. (2021). Phytochemistry and Pharmacology of Moringa Tree: An overview. Biointerface Res. Appl. Chem., **11**(3):10776-10789.

Garg, P. and Garg, R. (2018). Qualitative and quantitative analysis of leaves and stem of *Tinospora cordifolia* in different solvent extract. J. Drug Deliv. Ther., **8**(5-s):259-64.

Goyal, A.K. (2014). Phytochemical and medical properties of *Ficus religiosa* specific parts extracts: A review. International J. Pharm. Life Sci., **5**(4):3424-3429.

Gregory, M.; Divya, B.; Mary, R.A.; Viji, M.M.; Kalaichelvan, V.K. and Palanivel, V. (2016). Antiulcer activity of *Ficus religiosa* leaf ethanolic extract. Asian Pac. J. Trop. Biomed., **3**(7):554-556. Doi:10.1016/S2221-1691(13)60112-4

Harborne, J.B. (1984). Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall.

Khandelwal, K. R. (2000). Practical pharmacognosy. 2nd ed. Nirali Prakashan. India: Pune.

Kirtikar, K.R. and Basu, B.D. (1993). Indian medicinal plants. Periodical Experts Book Agency. India: New Delhi.

Kokate, C.K. and Gokhale, S.B. (2008). Practical pharmacognosy. Nirali Prakashan. India: Pune., pp:34-49.

Koilpillai, B.; Sabesan, G. S. and Sadananda, R. (2010). Comparative pharmacognostic studies on the barks of four Ficus species. Turk. J. Bot., **34**:215-224.

Krishnaiah, D.; Devi, T.; Bono, A. and Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plant Res., **3**(2):67-72.

Krishnarao, K.L. and Raja Rajeswari, T. (2023). investigation and evaluation of the antioxidant, antibacterial and antifungal activities of *Stephania japonica* L. leaves extract Ann. Phytomedicine., **12**(1):477-485.

Mahajan, S.G; Mali, R.G. and Mehta, A.A. (2007). Effect of *Moringa oleifera* Lam. seed extract on toluene diisocyanate-induced immune-mediated inflammatory responses in rats. J. Immunotoxicol., **4**(2):85-96.

Manu, K.A. and Kuttan, G. (2009). Immunomodulatory activities of punarnavine, an alkaloid from *Boerhavia diffusa*. Immunopharm. Immunot., **31**(3):377-387.

Marcocci, L.; Maguire, J.J.; Droylefaix, M.T. and Packer, L. (1994). The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem. Biophys. Res. Comm., **201**(2):748-755. Doi:10.1006/bbrc.1994.1764

Mishra, A. and Khushtar, M. (2021). Phytochemical estimation of germinated *Trigonella foenum-graecum* L. seed extract for better application in phytotherapy Sahar Idris, Ann. Phytomedicine., **10**(2):213-222.

Mohanasundar, C.; Natarajan, D.; Srinivasan, K.; Umamaheswari, S. and Ramachandran, A. (2007). Antibacterial properties of *Passiflora foetida* L. A common exotic medicinal plant. Afr. J. Biotechnol., **6**(23):2650-3.

Nair, R.; Kalariya, T and Sumitra, C. (2005). Antibacterial activity of some selected Indian medicinal flora. Turk. J. Bot., **29**:41-7.

Nayak, P and Thirunavoukkarasu, M. (2016). A review of the plant *Boerhavia diffusa*: Its chemistry, pharmacology and therapeutical potential. J. Phytopharm., **5**(2):83-92.

Patil, K.S. and Bhalsing, S.R. (2016). Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus *Boerhavia*. J. Ethnopharmacol., **182**:200-220. Doi:10.1016/j.jep.2016.01.042

Pawaskar, S.M. and Ranade, K.J. (2022). Phytochemical analysis and *in vitro* antioxidant studies of the leaf extracts of some Indian medicinal plants from western Maharashtra Ann. Phytomedicine, **11**(2):507-515.

Prasad, P.V.; Subhakthe, P.K.; Narayana, A. and Rao, M.M. (2006). Medico historical study of "Asvattha" (Sacred Fig Tree). Bull. Indian Inst. Hist. Med., Hyderabad, **36**:1-20.

Rajshekharan, P.E. (2002). Herbal medicine. In: World of Science. Employment News., **3**. pp:21- 27.

Rani, N.Z.A.; Hussain, K. and Kumolosasi, E. (2018). *Moringa* Genus: A review of phytochemistry and pharmacology. Front. Pharmacol., pp:1-26.

Sharma, A.; Gupta, A. and Singh, S.A.B. (2010). *Tinospora cordifolia* (Willd.) Hook. F. and Thomson: A plant with immense economic potential. J. Chem. Pharm. Res., **2**(5):327-333.

Sharma, P.; Dwivedee, B.P.; Bisht, D.; Dash, A.K. and Kumar, D. (2019). The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon., **5**(9):1-8.

Sharma, R. and Singh, V.J. (2010). *In vivo* antioxidant activity of *Moringa oleifera* leaf and pod extracts against carbon tetra chloride induced liver damage in albino mice. J. Chem. Pharm Res., **2**(6):275-283.

Sharma, V.; Mishra, S.; Yesudas, R and Rajput, R. S. (2019). A review on *Ficus religiosa* (Sacred Fig). International J. Res. Anal. Rev., **6**(2):901-906.

Singh, D.; Singh, B. and Goel, R. K. (2011). Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. J. Ethnopharmacol. **134**(3):565-583. Doi:10.1016/j.jep.2011.01.046

Singh, R.H and Udupa, K.N. (1972a). Studies on the Indian indigenous drug, Punarnava (*Boerhavia diffusa* Linn.) Part II. Preliminary phytochemical studies. J. Res. Edu. Indian Med., **7**:13-16.

Sinha, K.; Mishra, N.P.; Singh, J. and Khanuja, S.P.S. (2004). *Tinospora cordifolia* (Guduchi), A reservoir plant for therapeutic application. Indian J. Tradit. Knowl., **3**(3):257-70.

Sivarajan, V.V. and Balachandean, I. (1999). Ayurvedic drugs and their plants sources, Oxford and IBH Publishing. New Delhi, India.

Thakur, R.S.; Soren, M.G; Pathapati, R.M. and Buchineni, M. (2016). Diuretic activity of *Moringa oleifera* leaves extract in swiss albino rats. J. Pharm. Innov., **5**: 8-10.

Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G. and Kaur, H. (2011). Phytochemical screening and Extraction: A review. Int. Pharm. Sci., **1**(1):98-106.

Upadhyay, A.; Kumar, K.; Kumar, A. and Mishra, H. (2010). *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi) - validation of the ayurvedic pharmacology through experimental and clinical studies. Int. J. Ayurveda Res., **1**(2):112-125. Doi:10.4103/0974-7788.64405

Venkatachalam, T.; Chitra, M.; Kalaiselvi, P.; Chitra, A.; Sumathi, K.; Babu, C.M.S.; Senthilkumar, N. and Sattanathan, K. (2021). Phytochemical screening and antidiabetic potentiality of *Pavetta indica* L. (Angiosperms: Rubiaceae) methanol extract on streptozotocin induced diabetic mice. Ann. Phytomed., **10**(2):292-297.

Zhishen, J.; Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., **64**(4):555-559. Doi:10.1016/s0308-8146(98)00102-2

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