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Phytochemical Screening, Total Phenolics and Antioxidant Activities of Selected 100 Medicinal Plants of Semi-arid Region of Gujarat, India

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ABSTRACT

Phytochemicals are bioactive compounds obtained from plants and widely applied for herbal medicine preparation. Plants are a source of many drugs such as antispasmodics, emetics, antimicrobials, antipyretics, antidiarrheals, antioxidants, and antitumor agents. Traditional medicine involves the use of different plant extracts or bioactive constituents. Hence in the present study, one hundred medicinal plants were collected from the Saurashtra region, Rajkot, Gujarat. The extract was prepared from the leaf and screened for tannins, coumarins, saponins, steroids, phenol, flavonoids, and alkaloids like phytoconstituents. Further extracts were used for the total phenolic content and antioxidant activities estimation. In all plant extracts, phenol and tannin are present. Antioxidant activity was determined by DPPH radical scavenging methods. From the studied plants many selected species have high levels of phenolics and exhibit high antioxidant capacity. The results concluded that the studied plants are rich in phytochemicals with significant pharmacological and medicinal applications. The probable role of these compounds with medicinal properties is discussed.

Keywords: Medicinal plant, Phytochemical, Antioxidant activity, Phenolic content

INTRODUCTION

Medicinal plants are important effective sources of both traditional and modern medicine. The global economy's importance of the international trade of herbal products and the alternative medicine market has been increasing by approximately 15% annually [1, 2]. Approximately 29,000 herbal substances used by more than 1000 companies have annual revenues of over US$60 billion, with the bulk of the herbal products being sourced from biodiversity-rich countries in Asia, Africa, and South America [3, 4]. In India, 70-80% of the population depends on medicinal herbs [5]. In India, approximately 80 % of medicinal plant species are either cultivated or collected from the wild [6]. These plants are used for the treatment of various diseases in different parts of the world. India has become the largest exporter country of medicinal plants, herbal drugs, and their related products in the world. The export value of Indian medicinal plants and their related products is estimated at around US$110 billion and the annual turnover of medicine is about US$1 billion and growing [7]. There are nearly 9000 traditional pharmaceutical manufacturers company in India, and most of them are involved in the formulation of Ayurvedic drugs [7].

In a veritable emporium of medicinal and aromatic plants of India, more than 25,000 plant species are known for their applications in various fields. Around 9000 are commonly useful, 7,500 (medicinal), 3900 (edible), 700 (culturally important), 500 (fiber), 300 (pesticide and insecticide), 300 for resin, gum, and dye, and 100 for perfume [8]. Medicinal plants play a major role in almost all the traditional systems of medicine. India has unique six recognized systems of medicine. They are Ayurveda, Siddha, Unani and Yoga, Naturopathy, and Homoeopathy [9]. Medicinal plants are rich sources of novel drugs that form the ingredients in traditional systems of medicine, modern medicines, food supplements, and, pharmaceutical intermediates [10]. Medicinal plants have many biological effects on the human system including antioxidant activity due to the presence of various compounds such as flavonoids, phenolic acids, tannins, coumarins, lipids, and lignins in different parts of a plant [11]. The main aim of the present research was to study the screening of qualitatively phytochemical content and possible estimation of phenolic contents and antioxidant activities of 100 medicinal plants of the Saurashtra region. The potential of antioxidant activities and phenolic content of the extract from these plants are of great interest in the food and pharmaceutical industry.

MATERIAL AND METHODS

Plant material

A total of 100 medicinal plant leaves were collected from the Saurashtra region, Rajkot, Gujarat, India.

Extraction of plant material

Healthy leaves were used for the sample preparation, each was washed under running tap water several times and rinsed with distilled water. It was dried over filter paper and 72h of oven dry. After completion of the drying process, the material was ground in a grinder and the powder was kept in appropriately labeled plastic bags. One gram of ground material was weighed using an electronic weighting balance, the sample was homogenized with a mortar and pestle containing 20 ml of methanol. Then keep them in 48h of dark condition. The samples were centrifuged at 8000 rpm for 10 minutes. The pellets were discarded and supernatants were pooled and used for the antioxidants, phenol estimation, and phytochemical test analysis.

Phytochemical analysis

Preliminary qualitative screening for phytochemicals, of all these plant species was carried out with the following methods.

Test for Tannins

2 ml of the extract was added with a few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins [12].

Test for Coumarins

2 ml of extract was treated with 3 ml of 10% NaOH. Observed the formation of yellow color indicating the presence of coumarins [13].

Test for Saponins (Foam test)

2 ml of extract was taken in a test tube and 6 ml of distilled water was added to it. The mixture was then shaken vigorously. The persistence of foam was observed that indicating the presence of saponins [13].

Test for Steroids (Libermann Burchard Test)

1 ml of extract was dissolved in 1 ml of chloroform. To this mixture, an equal volume of concentrated sulfuric acid was added by the sides of the test tube. The upper layer becomes red while the lower layer of sulfuric acid turns yellow with green fluorescence indicating the presence of steroids [13].

Test for Phenolic Compounds (Ferric chloride test)

1 ml of extract was taken in a test tube and 0.2 ml Folin reagent and sodium bicarbonate was added to it. The formation of a deep blue or black color indicates the presence of phenolic compounds [14].

Test for Flavonoids (Alkaline reagent test)

2 ml of extract was treated with a few drops of 1N sodium hydroxide solution and the formation of an intense yellow color. This yellow color becomes colorless with the addition of dilute hydrochloric acid, indicating the presence of flavonoids [14] .

Test for Alkaloids

(a) Mayer's test

A one ml of plant sample extract, and two drops of Mayer's reagent are added along the sides of the test tube. The appearance of a white creamy precipitate indicates the presence of alkaloids.

(b) Wagner's test

A few drops of Wagner's reagent are added to a few ml of plant extract along the sides of the test tube. A reddish-brown precipitate confirms the test as positive [15].

Extraction of Phenolic compound from the Plant

One gram dry powder of leaves was taken and to this 10ml of 80% Methanol was added. The mixture was kept in the dark for 48 hours under shaking conditions. The next day the extracts were centrifuged at 8000g for 10 min. The supernatant was collected in a Petri plate and allowed for evaporation. The remaining dry residue was dissolved in sterile distilled water (5ml) and used as a source of phenols.

Total Phenolic content

The concentration of total phenols was determined according to Swain and Hills [16]. The reaction mixture contained 100µl of extract, 10µl of 1N Folin-Ciocalteaus reagent, and 200µl of saturated sodium carbonate. The reaction mixture was incubated at room temperature for 5 minutes. The absorbance was measured at 660 nm using Biotek microplate reader µ-Quant. Measurements were carried out in triplicate and phenol content was calculated using a calibration curve of gallic acid (100-1000µg/ml).

DPPH free radical scavenging assay

Radical scavenging assay was determined according to Valazquez et al., [17] with some alterations. DPPH (1,1-diphenyl-2- picrylhydrezyl) free radical scavenging assay is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds Bhalodia [18]. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. This transformation results in a color change from purple to yellow, which is measured on a microplate reader (µ Quant, Bio Teak, USA). The disappearance of the purple color is monitored at 517 nm. The reaction mixture (200µl) consists of 100µl of DPPH (1 mM) and 100µl methanol extract. It is incubated for 20 minutes at room temperature in the dark and then the absorbance is measured at 517 nm. The activity was expressed as mg equivalent to ascorbic acid/g dry powder, the experiment was performed in triplicate.

RESULTS AND DISCUSSION

In the present study, 100 medicinal plants were screened for the presence of medicinal important phytochemical constituents. A total of seven tests were performed for phytochemical analysis i.e. tannins, coumarins, saponins, steroids, phenol, flavonoids, and alkaloids. Total phenol and antioxidant activity were estimated from all selected plants.

The research work was carried out on the 100 selected medicine plants which show the phytochemical constituents i.e., tannins, coumarins, saponins, steroids, phenol, flavonoids, and alkaloids are either present or absent in these plants, and the results were summarized in Figure 1 and 2. The result of the phytochemical analysis shows that the one hundred plants are rich in at least one tannin, steroids, coumarins, saponins, phenol, flavonoids, and alkaloids. Plant *C. calendars, L. aspera, C. papaya, J. gossypiifolia, X. strumarium, C.limon, A. excelsa, T. foenum-graecum, G. bonduc, V. negundo, N. cadamba, E. grandis, B. vulgaris, C. dichotoma, S. mukorossi, T. stans, D. regia, T. populnea, B. lacera, M. azedarach, T. cordifolia, T. catappa, A. bracteolata, B. spectabilis* and *P. betle* having all these

phytochemicals. This study has revealed the presence of phytochemicals considered active medicinal chemical constituents of these plants.

Tannins were present in 39 families of the plants. In Acanthaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apiaceae, Apocynaceae, Asteraceae, Bignoniaceae, Brassicaceae, Caesalpinioideae, Caricaceae, Celastraceae, Combretaceae, Crassulaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Lythraceae, Malvaceae, Meliaceae, Moraceae, Moringaceae, Myrtaceae, Nyctaginaceae, Phyllanthaceae, Piperaceae, Poaceae, Rubiaceae, Rutaceae, Salvadoraceae, Sapindaceae, Simaroubaceae, Solanaceae, Verbenaceae, and Zygophyllaceae family plants tannins were present in higher amount while Aristolociaceae, Boraginaceae, Menispermaceae, and Vitaceae family plants tannins were present in a lower amount.

Coumarins were present in 37 families of the plants. In Anacardiaceae, Apocynaceae, Aristolochiaceae, Bignoniaceae, Boraginaceae, Caesalpinioideae, Lythraceae, Myrtaceae, Phyllanthaceae, Rubiaceae, Sapindaceae, and Zygophyllaceae family plants coumarins were present in higher amounts. Caesalpinioideae family plants *C. tora, C. fistula, C. pulcherrima, S. alexandrina,* and *G. bonduc* showed a higher amount of coumarins present. In Amaranthaceae, Annonaceae, Apocynaceae, Asteraceae, Brassicaceae, Caricaceae, Celastraceae, Combretaceae, Crassulaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Meliaceae, Menispermaceae, Moraceae, Moringaceae, Myrtaceae, Nyctaginaceae, Piperaceae, Poaceae, Rutaceae, Salvadoraceae, Simaroubaceae, Solanaceae, and Verbenaceae family plants coumarins was present in lower amount while Acanthaceae and Vitaceae family plants coumarins was absent.

Saponin was present in 34 families of plants. In the Boraginaceae family, *C. dichotoma* plant and Sapindaceae family *S. mukorossi* plants show a higher amount of saponins were present. In Acanthaceae, Apocynaceae, Aristolochiaceae, Asteraceae, Combretaceae, Fabaceae, Lamiaceae, Lythraceae, Meliaceae, Menispermaceae, Moraceae, Myrtaceae, Nyctaginaceae, Poaceae, Rubiaceae, Rutaceae, Simaroubaceae, Verbenaceae, and Vitaceae family plants saponins were present in moderate amounts while Amaranthaceae, Annonaceae, Bignoniaceae, Brassicaceae, Caesalpinioideae, Caricaceae, Celastraceae, Euphorbiaceae, Lamiaceae, Malvaceae, Moraceae, Phyllanthaceae, Piperaceae, Rubiaceae, and Solanaceae family plants saponin was present in a lower amount. *M. indica* of the Anacardiaceae family, *C. sativum* of the Apiaceae family, *B. pinnatum* of the Crassulaceae family, *M. oleifera* of Moringaceae family, and *B. aegyptica* of the Zygophyllaceae family plants saponin was absent.

The steroid was present in 35 families of the plants. In Bignoniaceae, Nyctaginaceae, Piperaceae, Rubiaceae, Sapindaceae, and Zygophyllaceae family plants steroids were present in high amounts while Acanthaceae, Amaranthaceae, Annonaceae, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caesalpinioideae, Crassulaceae, Euphorbiaceae, Fabaceae, Lythraceae, Malvaceae, Menispermaceae, Moraceae, and Phyllanthaceae family plants steroids were present in a lower amount. The steroid was present in moderate amounts in Anacardiaceae, Aristolochiaceae, Caricaceae, Combretaceae, Lamiaceae, Meliaceae, Myrtaceae, Poaceae, Rutaceae, Simaroubaceae, Solanaceae, and Verbenaceae family plants. *G. montana* of Celastraceae family, *S. persica* of Salvadoraceae family, and *C. quadrangularis* of Vitaceae family plants steroids were absent.

Phenol was present in 39 families of plants. In Anacardiaceae, Annonaceae, Apocynaceae, Aristolochiaceae, Asteraceae, Bignoniaceae, Boraginaceae, Brassicaceae, Caesalpinioideae, Caricaceae, Celastraceae, Combretaceae, Crassulaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Lythraceae, Malvaceae, Meliaceae, Menispermaceae, Moraceae, Moringaceae, Myrtaceae, Nyctaginaceae, Phyllanthaceae, Piperaceae, Poaceae, Rubiaceae, Rutaceae, Sapindaceae, Verbenaceae, and Zygophyllaceae family plants phenol was present in highest amounts while Salvadoraceae, Simaroubaceae, Solanaceae, and Vitaceae family plants phenol were present in moderate amount. The phenol was present lowest amounts in Acanthaceae, Amaranthaceae, and Apiaceae family plants.

Flavonoid was present in 36 families of plants. In Anacardiaceae, Apocynaceae, Aristolochiaceae, Bignoniaceae, Boraginaceae, Caesalpinioideae, Celastraceae, Combretaceae, Crassulaceae, Moringaceae, Myrtaceae, Phyllanthaceae, Rubiaceae, Salvadoraceae, and Sapindaceae family plants flavonoid was present in the highest amount while Amaranthaceae, Annonaceae, Brassicaceae, Caricaceae, Euphorbiaceae, Fabaceae, Lythraceae, Meliaceae, Menispermaceae, Moraceae, Nyctaginaceae, Piperaceae, Poaceae, Solanaceae, and Zygophyllaceae family plants flavonoids were present in moderate amount. Flavonoids were present in a lower amount of Apiaceae, Asteraceae, Lamiaceae, Malvaceae, Rutaceae, and Simaroubaceae family plants. *L. camera* of the Verbenaceae family, *C. quadrangularis* of the Vitaceae family, *A. vasica*, *B. lupulina,* and *P. carruthersii* plants of the Acanthaceae family flavonoid were absent.

Alkaloid was present in 33 families of plants. In Acanthaceae, Apocynaceae, Aristolochiaceae, Bignoniaceae, Boraginaceae, Caricaceae, Piperaceae, Verbenaceae, and Zygophyllaceae family plants alkaloid was present in higher amounts while Caesalpinioideae, Fabaceae, Moringaceae, Rubiaceae, Rutaceae, Sapindaceae, Simaroubaceae, and Solanaceae family plants alkaloid were present in moderate amount. Alkaloids were present in the lower amount of Amaranthaceae, Apiaceae, Asteraceae, Combretaceae, Euphorbiaceae, Lamiaceae, Lythraceae, Malvaceae, Meliaceae, Meliaceae, Menispermaceae, Moraceae, Myrtaceae, Nyctaginaceae, Phyllanthaceae, Poaceae, and Vitaceae family plants. In Anacardiaceae, Annonaceae, and Salvadoraceae family plants alkaloid was absent.

In our study, it was investigated that the 25 plants having all these phytochemicals such as *C. carandas, L. aspera, C. papaya, J. gossypiifolia, X. strumarium, C. limon, A. excelsa, T. foenum-graecum, G. nonfiction, V. negundo, N. cadamba, E. grandis, B. vulgaris, C. dichotoma, S. mukorossi, T. stans, D. regia, T. populnea, B. lacera, M. azedarach, T. cordifolia, T. catappa, A. bracteolata, B. spectabilis,* and *P. betle*. In the present work tannins and phenol are present in all selected plant samples. According to the previous literature, tannins are reported to have cardio-protective, anti-inflammatory, anti-carcinogens, and antimutagenic properties [19].

Tannin is also involved in the treatment of non-insulin-dependent diabetes mellitus by enhancing glucose uptake and inhibiting adipogenesis [20]. The results of our study have shown that phenolic compounds are present in all samples. Phenolic compounds are known for their role in regulating the immune system, their anti-inflammatory effect, chemoprevention, neuro protection, cardio protection, and in the treatment of diseases such as diabetes, Parkinson's, and cancer; in addition to this, they also have antibacterial and antiviral effects [21, 22]. In this study, the highest amount of tannins, coumarins, saponins, steroids, phenol, flavonoids, and alkaloids were present in *B. spectabilis*. Previous literature has reported that *Bougainvillea* contains several phytochemicals such as quinones, saponins, triterpenoids, flavonoids, phenol, sterols, glycosides, tannins, furanoids and a small number of sugars [23]. According to the literature, saponins exhibit various biological activities, They give permeability to the cell membrane, help lower serum cholesterol levels, possess abortifacient properties, it have immunomodulatory properties [24].

Plant steroids possess many interesting medicinal pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, anthelmintic, cytotoxic, and cardiotonic activity [25]. In the present work, steroids were absent in the *C. roseus* of the Apocynaceae family plant, while the previous research study showed that steroids were present in it[26]

In our recent research studies tannins and saponins were present in *D. regia* and *T. foenum-graecum* plants of the Fabaceae family, while previous literature has reported that tannins and saponins were absent in it [27]. The recent research studies and previous research studies results were different so it might be due to the change in location and genetic variation due to cross-pollination, so their genetic makeup was changed and that is why shows the different results. The phytochemical analysis of *A. precarious* and *M. pinnata* showed the absence of steroids and coumarins, while the previous study showed that the steroid and coumarins were present [27]. Coumarins, a major class of flavonoids, possess pharmacological properties like antidiabetic, antioxidant, hepato-protective, anticoagulant, antimicrobial, anti-inflammatory, analgesic, anticancer, antiviral, antimalarial activities, etc [28, 29]. In the present study, according to the phytochemical analysis of *L. aspera*, *H. suaveolens* and *O. basilicum* plants of the Lamiaceae family saponin was present, while the previous literature has reported that saponin was absent in it [30].

Flavonoids were found to be present in *E. heterophylla* and *E. hirta* plants of the Euphorbiaceae family according to the previous investigation, while in the present investigation, flavonoids were absent in *E. heterophylla* and *E. hirta*. From the literature, survey flavonoids have a wide range of biological properties such as anti-inflammatory, antibacterial, antiviral, anti-allergic, and cytotoxic antitumor properties [31]. It is used in the treatment of neurodegenerative diseases and has vasodilatory action [32].

Radical scavenging activity was measured in mg/g dry powder and measured by the DPPH free radical scavenging method. The stable free radical DPPH was reduced and decolorized. The higher free radical scavenging was observed in 32 plants such as *T. cordifolia* (150.24 mg), *P. carruthersii* (149.91 mg), *B. erecta* (149.77 mg), *C. citratus* (149 mg), *B. lacera* (148.8 mg), *C. dichotoma* (148.73 mg), *D. innoxia* (147.64 mg), *T. arjuna* (147.6 mg), *S. oleraceus* (147.44 mg), *V. negundo* (146.8 mg), *B. vulgaris* (144.71 mg), *B. aegyptica* (143.88 mg), *B. monosperma* (143.73 mg), *R. communis* (143.62 mg), *T. catappa* (141.95 mg), *M. piperita* (141.6 mg), *C. multiflorum* (141.6 mg), *M. charantia* (140.93 mg), *T. erecta* (140.82 mg), *E. grandis* (140.02 mg), *N. cadamba* (139.88 mg), *D. regia* (139.55 mg), *P. betle* (137.55 mg), *A. excelsa* (137.51 mg), *S. mukorossi* (136.68 mg), *A. indica* (134.55 mg), *P. hysterophorus* (134.42 mg), *C. limon* (134.06 mg), *A. bracteolata* (131.31 mg), *G. bonduc* (130.95 mg), *S. cumuni* (130.88 mg), *J. gossypifolia* (130.48 mg) (Fiure 3, 4). A moderate amount of antioxidant activity was observed in many plants. Antioxidant activity was a minimum range below 130 to 50 mg/gm dry powder. Antioxidant activity was observed minimum amount in two plants such as *B. pinnatum* (47.84 mg) and *L. camera* (42.93 mg). In our recent research studies, higher antioxidant activity was observed in *T. cordifolia*, *P. carrutherssi*, *B. erecta,* and *C. citratus* plants. In several studies, it is reported that the antioxidant properties of herbal materials are of great value for pharmaceutical properties. Antioxidant activity, including the radical scavenging and redox potential of polyphenols, is generally thought to be a reason behind their beneficial effect on human health [33]. Based on *in vitro* assays, the effects comprise neuroprotective, cardioprotective, anti-inflammatory, anti-cancer, and antibacterial properties [34].

In this work, the total phenols, contents of all the selected medicinal plants were expressed as mg/gm dry powder. The total phenol contents of all plants are presented in Figures 5 and 6. We have observed that the maximum phenolic concentration in 15 plants such as *C. pulcherrima* (229.58 mg), *F. benghalensis* (221.40 mg), *M. charantia* (195.44 mg), *V. negundo* (189.85 mg), *A. scholaris* (182.20 mg), *L. aspera* (175.98 mg), *P. guajava* (173.92 mg), *D. regia* (171.85 mg), *E. milii* (169.50 mg), *P. betle* (163.92 mg), *D. innoxia* (162.58 mg), *B. aegyptica* (159.62 mg), *T. stans* (159.04 mg), *S. mukorossi* (158.92 mg) and *N. cadamba* (150.22 mg). Phenol was detected in the middle range of 30 to 150 mg/gm dry powder in many plants. The lower concentration was detected in three plants such as *B. pinnatum* (29.72 mg), *P. peruviana* (27.41 mg), and *H. suaveolens* (26.50 mg). In the present work, higher phenolic content was detected in *C. pulcherrima* and *F. benghalensis* plants. Phenolic compounds possess biological properties such as apoptosis, anti-aging, anticarcinogenic, anti-inflammation, anti-atherosclerosis, cardiovascular protection, and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activity [35].

CONCLUSION

Overall, from this study, it is concluded that all selected plants are rich in phytochemicals constitute, have pharmacological and medicinal importance. Out of all secondary metabolites tannin and phenol are observed maximum in almost all plant species. Most of all selected plant species are given a higher amount of antioxidant and phenolic content. These plants will be used as a source of natural antioxidants. However, further study is required to find their biological activities.

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Figure -1 : Phytochemical analysis of selected medicinal plant species

Note : score 0: absent; 1: lowest; 2; moderate; 3: higher activity

Figure -2 : Phytochemical analysis of selected medicinal plant species

Note : score 0: absent; 1: lowest; 2; moderate; 3: higher activity

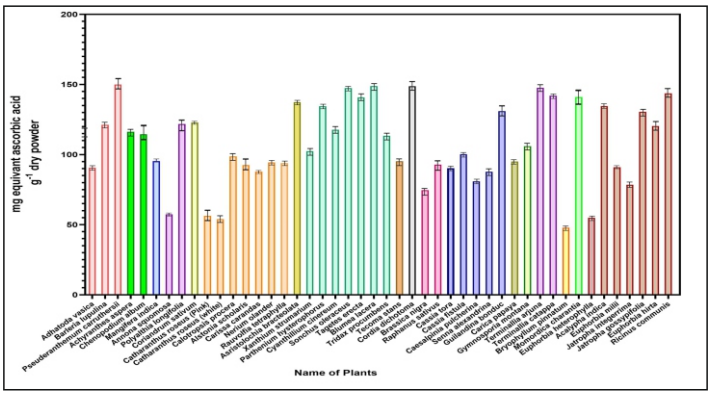

Figure -3: Antioxidant activity of selected medicinal plants

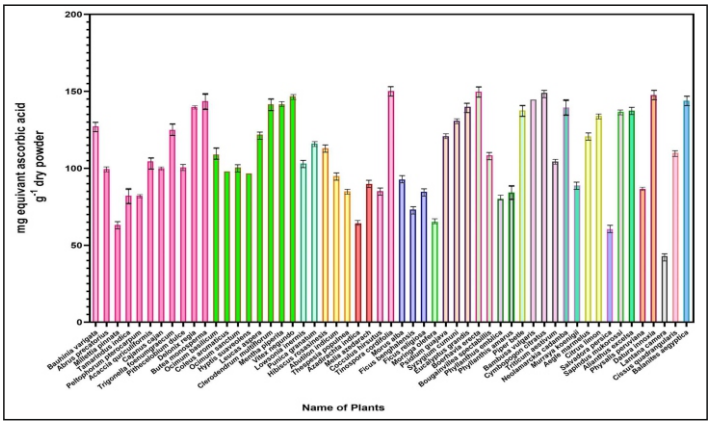

Figure -4: Antioxidant activity of selected medicinal plants.

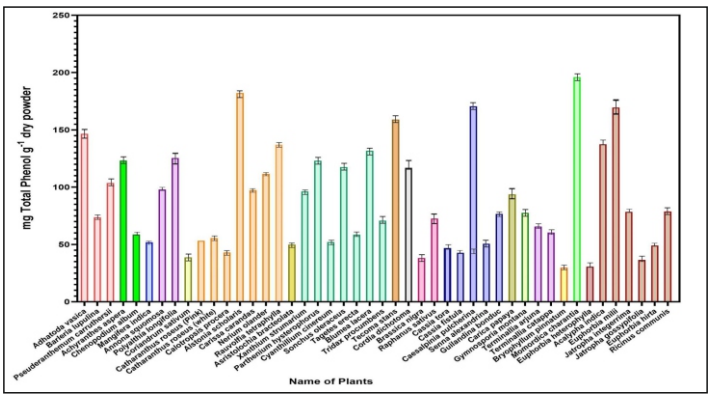

Figure -5: Total phenolic contents of selected medicinal plants

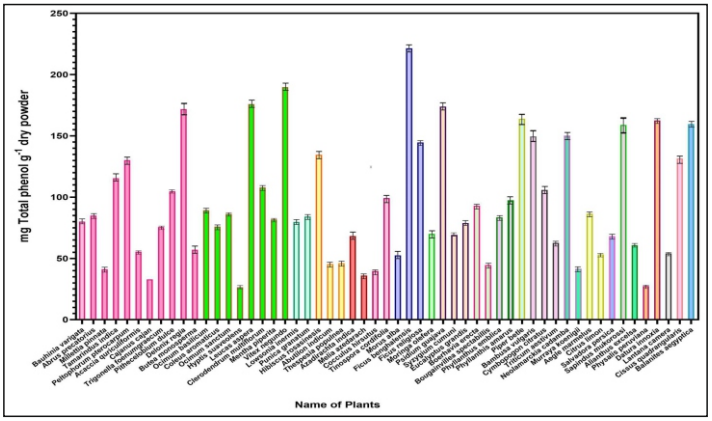

Figure- 6: Total phenolic contents of selected medicinal plant

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