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Research Article

Phytochemical Investigation and Antioxidant Activity of Ethnomedicinal Plants from Ratanmahal and Udalmahuda Forest, Dahod

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ABSTRACT

Tribal people of Ratanmahal and Udalmahuda forest have been using more than 17 plants to treat various ailments. The main aim of the study is to investigate the medicinal properties of the ethnomedicinal plants i.e., *Buchanania lanzan* Sprang., *Millettia peguensis* Ali., *Evolvulus alsinoides* L. These plants are used for different remedies like improving sexual health, eczema & typhoid. Medicinal plants contain bioactive compounds known as phytochemicals. These phytochemical compounds are used in various medicines. This paper reports the phytochemical screening and antioxidant activity of Ethnomedicinal plants of *Buchanania lanzan* Sprang., *Millettia peguensis* Ali., *Evolvulus alsinoides* L. collected from Ratanmahal and Udalmahuda forest, Dhanpur, Dahod, Gujarat. Quantitative estimation of phenol was done by Folin-ciocaltue colorimetric method and total flavonoid content was determined by the Aluminium chloride ($AlCl_3$) colorimetric method. The Free H^+ radicle scavenging potential of plant samples was determined by the DPPH scavenging assay. In addition, the antioxidant potential of the samples was also determined by FRAP assay. In the current study, we can say that *B. lanzan* Sprang. Bark methanolic extract contains the highest phenolic content 2.554 mg GAE/g. while the methanolic extract of leaves of *Millettia peguenensis* contains the highest flavonoid content 10.262 mg QE/g. For the total flavonoid content, hexane extract of bark of *B. lanzan* contains the highest proportion of flavonoid 15.55 mg QE/g, on the other hand, methanolic solution of the leaves of *B. lanzan*, contains the highest content of flavonoid 7.4971 mg QE/ g. DPPH antioxidant activity demonstrates that methanolic extract of bark of *E. alsinoides* has the least IC_{50} value of 0.6346, the bark of *B. lanzan* has 0.7162 IC_{50} value, and leaves of *M. peguenensis* contain highest IC_{50} value 1.0508. From these, we can say that Bark of *E. alsinoides* has the highest ability to scavenge free H^+ radicals. From the FRAP assay of plant extract, methanolic extract of *B. lanzan* has the highest proportion of scavenging Fe^{+2} ions while hexane extract of *E. alsinoides* L. has the least proportion to scavenge Fe^{+2} ions. Due to the presence of secondary metabolic active compounds, it shows some biological and pharmacologic activity like Antioxidant, Anti-inflammatory, Antimicrobial, Anti-Fungal, and many more. The presence of potent Phytochemicals in the plants can be used in pharmaceuticals.

Keywords: Total flavonoid content, total phenolic content, antioxidant, DPPH, FRAP

INTRODUCTION

Natural products are made from different parts of plants that are being used by mankind millions of years ago. Tribal people of the forest area of India use traditional plants to cure various types of diseases[1].The forest patches of Orissa reported 46 indigenous plant species, which are being used by tribals to cure Gastrointestinal ailments, Skin diseases, Jaundice, Diabetes, Snakebite, and so on[2]. Ancient people from forest areas used these plants to cure various kinds of diseases[3]. Twenty-one ethnomedicinal plants are reported from Jhalod forest, Dahod, Gujarat. These plants are used as medicine to cure various ailments[4]. Plants produce chemicals in order to protect themselves, these chemicals are known as phytochemicals[5]. Phytochemicals are metabolically active compounds. They promote plant growth, giving protection against various kinds of infectious agents like fungi, bacteria, and viruses. To isolate different chemicals from plants and to determine their structure, in vivo and invitro method is generally used[6]. Phytochemicals can be classified into two groups (1) Primary metabolites and (2) Secondary metabolites. Proteins, Sugars, Amino acids, Purines, Pyrimidines, and Nucleic acids are considered Primary metabolic compounds. While alkaloids, phenols, flavonoids, and tannins are the type of secondary phytochemicals[7]. The amount of phytochemicals varies from plant parts as well as species to species. Their presence and their total amount can be determined by qualitative and quantitative analysis. Generally, in leaves, alkaloid is present in very low concentration as compared to the phenolic compound. Whereas roots, fruits, and seeds are rich in alkaloid compounds[8]. Alkaloids and glycosides are complex chemical substances and are distributed in large varieties of plants throughout the plant kingdom. Many of these alkaloids and glycosides are poisonous but they can be used in medicines if given in optimum dosage[9] According to studies, free radicals play an important role in affecting human health by causing a variety of chronic diseases, including cancer, diabetes, aging, atherosclerosis, hypertension, heart attacks, and other degenerative diseases[9]. These free radicals are generated during metabolism. Exogenous intake of antioxidants can

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help the body to scavenge free radicals effectively[10]. Two kinds of antioxidants are found i) Endogenous ii) Exogenous. For gaining the highest cellular function, antioxidants will scavenge the free radicals in the human body[12]. Free radicals cause damage to cells and tissue, antioxidants will donate the electron to stabilize the ROS[13]. *Buchanania lanzan*, generally grown in tropical regions of Chhattisgarh, Jharkhand, Madhya Pradesh, Varanasi, and Mirzapur districts of Uttar Pradesh, it was first described in 1798 by Francis Hamilton[14]. Seven species of this plant have been reported in India, among them, *B.lanzan* and *B.axillaris* produce edible fruit[15,16]. It is a commercially useful tropical plant, growing up to 50 ft tall[17]. It bears fruits each containing a single seed, known as chironji. The plant is used in various ways for the treatment of many diseases. Oil extracted from the seeds is used to reduce swelling of the neck[18,19]. Oil from the kernel relieves the itch and prickly heat. The gum extracted from the bark helps treat diarrhoea and intercostal pains. The leaf paste encourages wound healing[20]. Ethanolic and methanolic extract of the root of *B. lanzan* Sprang has significant wound-healing activity[21]. Ethanolic extract of *Buchanania lanzan* Spreng barks reduced chromosomal damage and oxidative stress[22]. Evidence has proven that the consumption of antioxidant-rich foods may prevent certain types of cancer and reduce the risk of cardiovascular and cerebrovascular events. Methanolic extract of leaves also contains triterpenoids, saponins, flavonoids, kaempferol 7-o'glucosides, quercetin 3-rahmnoglucoside, kaempferol, gallic acid, kaempferol, and reducing sugars, such as myricetin-3-rhmnoside-3-galactoside[17,23]. Besides carbohydrates, fibers, minerals, fats, vitamins B1, B2, B3, C, copper, iron, magnesium, phosphorus, potassium, sodium, sulphur, fatty oil, and *-amyrin, the seed, and oil contain a number of other compounds and nutrients[24]. More than 200 species of Millettia are found in tropical and subtropical climates across the world. Millettia belongs to the Fabaceae family[25]. Due to the presence of flavonoids and their derivatives in Millettia species, the species were shown to have antitumor, cardiovascular function, anti-estrogen, antioxidant, insecticidal, and piscicide properties[26-31]. *Evolvulus alsinoides* L. is an annual herb from the family Convolvulaceae. It has a woody, branched rootstock[32]. Since the ancient time period, people from East Asia, India, Africa, and the Philippines are using this plant to cure fever, cough, cold, venereal illnesses, azoospermia, adenitis, and dementia[33]. It is also used to enhance memory power[34]. Evidently, this plant shows neurodegenerative disorders, antioxidant activity, anticonvulsant activity, antibacterial activity, antidiabetic activity, and anti-inflammatory activity[35]. Some Primary and secondary phytochemicals are biologically active in antioxidant, antimicrobial, increase immune system, etc[36]. Around 4500 phytochemicals are studied and among these 350 phytochemicals are studied in detail for their functions, chemical reaction, and their physical structure[37]. Therefore, the objective of this research work is to evaluate phytochemicals qualitatively and quantitatively and their ability to scavenge free hydroxyl radicle by DPPH antioxidant activity.

MATERIALS AND METHODS

Collection of Plant Materials:

Healthy plant parts leaves, bark, fruit, root of *Buchanania lanzan*, *Millettia peguenensis* and *Evolvulus alsinoides* were collected from Ratan Mahal and Udalmahuda forest, Dhanpur, Dahod on 17th October 2021. All plants were authenticated by Prof. Dr. Hitesh Solanki, Professor, Department of Botany, Gujarat University.

Preparation of Plant Extract

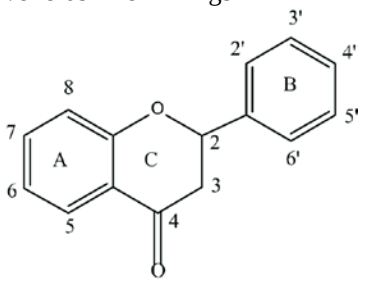
Plant parts were dried under shade and ground to powder form. Plant extracts were prepared by cold maceration.10gm of plant powder was weighed with 100ml of methanol and hexane and kept in an orbital shaker for 24hrs. The solutions were filtrated by filter paper. The filtrates were taken in Petri-plates and excess solvents were allowed to evaporate at room temperature. 30mg of dried plant extract was dissolved in 30 ml of methanol and hexane to prepare stock solutions. Then these stock solutions can be used for, Qualitative and quantitative analysis of phytochemicals (Total Phenolic and Total flavonoid Content), DPPH Antioxidant assay.

Quantitative test for Phytochemical Analysis

Total Flavonoid Content (TFC)

- *Working Principle:*

The aluminium chloride colorimetric method works on the basis that aluminium chloride forms acid-stable complexes with flavones and flavanols' C-4 keto group and either the C-3 or C-5 hydroxyl group. Aluminium chloride also forms acid-labile compounds with the ortho-dihydroxyl groups in flavonoids' A or B rings[38].



Basic Structure of flavonoid

- *Method:*

As the method mentioned by the Kariyone *et al.* (1953) [39]& Naghski *et al.* (1951)[40]. By slight modifications, total flavonoid content can be determined by $AlCl_3$. Took 1 ml plant extract, added 5ml of distilled water and 3ml of $NaNO_2$ and incubated it for 5 minutes. After the incubation period, added 3 ml of $AlCl_3$ and 1M NaOH. Afterward, make a final volume by addition of 10ml distilled water. Absorbance was measured at 510nm by a spectrophotometer.

An absorbance graph is plotted against the concentration of Quercetin. Based on the graph, regression equation (y = mx + c) will give us the value of phenolic compound in µg/ml. An unknown plant sample's flavonoid content can be calculated using the following formula:

QE equivalent = $\frac{C \times V}{m}$

QE= Quercetin equivalent (mg/ml)
V=Volume of plant extract

2.4 Total Phenol Content (TPC)

- *Working Principle:*

The Folin-Ciocalteu method is used in the phenolic quantification Assay. Phospho-molybdic/phosphor-tungstic acid complexes are present in the F-C reagent. The approach is based on the transfer of electrons from phenolic compounds to a blue chromophore made up of a phosphor-tungstic/phosphor-molybdenum complex in an alkaline solution, with the maximum absorption determined by the concentration of phenolic compounds. A spectrophotometer can detect the decreased Folin-Ciocalteu reagent in the range of 690 to 710 nm[41].. Plant extracts possess polyphenols that react with particular redox reagents (Folin Ciocalteu reagent) to generate a blue complex that can be measured using visible light spectrophotometry[42].

- *Method:*

Total phenolic content of *B. lanzan*, *M. peguenensis*, and *E. alsinoides* part extracts was determined by Folin ciocalteu reagent according to Donald *et al.*, (2001)[43]. 1 ml of plant extract was introduced into test tubes, and 500µl of distilled water and F-C reagent were mixed with plant extract. Incubate it for 5 minutes then add 1.5 ml 20% Na_2CO_3. Mixed it thoroughly and incubate it for 2 hr for a reaction period. Here methanol and hexane were used as a blank and gallic acid was used to produce a standard calibration curve. A standard graph is plotted against the concentration of gallic acid and its absorbance against 750 nm. Based on the graph, regression equation (y = mx + c) will give us the value of phenolic compound in µg/ml. Total phenolic content can be expressed as Gallic acid equivalent gm of dry weight (mg GAE/g) of extracts. The amount of phenolic content in an unknown plant sample can be determined by the following formula:

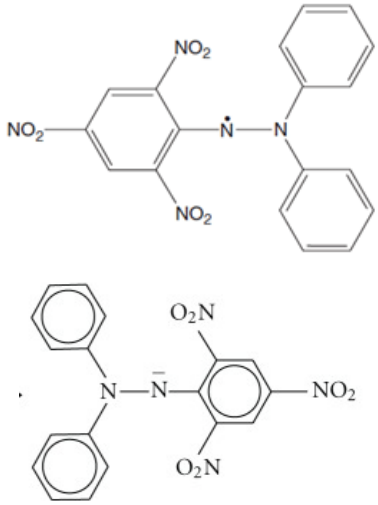
GAE Equivalent = $\frac{C \times V}{m}$

GAE = Gallic acid Equivalent (mg/ml)
V = Volume of plant extract *Determination of Antioxidant Capacity:*

DPPH Scavenging Activity:

- *Working principle:*

It is based on measuring the ability of antioxidants to scavenge free radicals. By receiving a hydrogen atom from antioxidants, the odd electron on nitrogen in DPPH is converted into the corresponding hydrazine[44].DPPH is characterized as a stable free radical due to the fact that the spare electron has been delocalized over the molecule. Additionally, delocalization causes the deep violet colour, with 520nm absorption in polar solutions. It loses its violet colour when DPPH solution is mixed with an atom donor. While DPPH can receive an electron or a hydrogen radical to form a stable, diamagnetic molecule, it can only be oxidized slowly and permanently. Because of its odd electron, DPPH has a significant absorption band at 517 nm, giving the solution a deep violet colour. However, once the electron pairs off, the absorption diminishes[45].



DPPH free radicle form & DPPH non radical form

- *Method:*

The crude extract and separated compounds' free radical scavenging activity were assessed according to Magalhaes *et al.*, (2006)[46]. Add methanol to 0.2, 0.4, 0.6, and 0.8 mL of plant extract to obtain a final volume of 1 ml. Then, 3 mL of a 0.004 % w/v methanol solution of DPPH was added. The reaction was then completed by keeping the tubes containing the mixture at room temperature for 30 minutes in a dark place. Using an ultraviolet-visible spectrophotometer, the absorbance was measured at 517 nm. Positive control was utilized, which was ascorbic acid.

I% Inhibition= $\frac{A0-A}{A0} \times 100$

Here, A_0 corresponds to the absorbance value of the blank sample, *A* value stands for absorbance of the sample. The final result was expressed as IC_{50} value[47.]

2.5 FRAP assay

- *Working principle:*

In an acidic media, the ferric reducing antioxidant power (FRAP) assay assessed the reduction of the ferric ion (Fe^{3+})-ligand complex to the strongly blue-coloured ferrous (Fe^{2+}) complex by antioxidants. Antioxidant activity is measured as a change in absorbance at 593 nm, and the results are represented as micromolar Fe^{2+} equivalents or as a percentage of an antioxidant standard[48]. Antioxidants can either reduce Fe^{3+} in the solution to Fe^{2+}, which binds the ferricyanide and produces Prussian blue, or they can reduce ferricyanide to ferrocyanide, which binds the free Fe^{3+} in the solution and produces Prussian blue. This is a simplified approach for these two reactions[49.]

Antioxidant+Fe^3 + ⇄ Fe^2 + +oxidized antioxidant
Fe^2 + +Fe (CN) 3 − 6 ⇄ Fe [Fe $(CN)_6$]

- *Method:*

A method based on this was developed by Benzie *et al.* (1996)[50]. Using this method, some unknown plant samples have been evaluated for antioxidant potential. Prepare FRAP solution by mixing 100ml of buffer 30mM of acetate, 10ml of buffer 10mM of TPTZ, and 10ml of buffer 20mM FeCl3 in equal parts. The ratio should be 10:1:1. Make 100ml of total volume by adding distilled water.

Prepare acetate buffer (30mM) by mixing 0.31 gm of sodium trihydrate with 1.608 ml of glacial acetic acid and diluting it with 100 ml of distilled water. 10 ml of 10 mM of TPTZ was prepared by weighing 0.031 g of TPTZ and diluting it into 10 ml of distilled water. In order to prepare a 20 mM 10 ml $FeCl_3$ solution, weigh 0.032 gm of fecl3 and dilute it in 10 ml of distilled water.

Prepare stand series of 200 µl -1000 ml. Here methanol and hexane were used as a blank and $FeSO_4$ was used to produce a standard calibration curve. A standard graph is plotted against the concentration of $FeSO_4$ and its absorbance against 593 nm. Final results were expressed as concentrations of antioxidants capable of reducing ferric in 1 gram of sample (l mg Fe^{+2}/g).

RESULT AND DISCUSSION

Total Flavonoid Content:

Estimation of Total Flavonoid from unknown plant samples was carried out by Aluminium Chloride Method. Table :1 shows the total content of phenols and flavonoids. *B. lanzan* leaves methanolic extract having higher amount of flavonoid (7.497 mg QE/g) while hexane extract having least flavonoid compound (4.225 mg QE/g). In *B. lanzan*, bark hexane extract has higher flavonoid content (15.556 mg QE/g) while methanolic extract of bark has the least flavonoid compound (7.336 mg QE/g). In *M. peguenensis*, leaves methanolic extract contains higher flavonoid compounds (10.262 mg QE/g.) while bark (5.249 mg QE/g) and fruit (7.272 mg QE/g) methanolic extract has fewer flavonoid compounds. Hexane extraction of *M. peguenensis* fruit has higher flavonoid content (6.318 mg QE/g) while leaves (5.165 mg QE/g) and bark (4.170 mg QE/g) hexane extract contain less flavonoid compound. In *E. alsinoides*, methanolic (15.411 mg QE/g) and hexane (7.082 mg QE/g) extract of the root has higher flavonoid content while leaves methanolic (5.411 mg QE/g) & hexane (5.975 mg QE/g) extraction and bark methanolic (7.059 mg QE/g) & hexane (4.401 mg QE/g) extraction contain least flavonoid content.

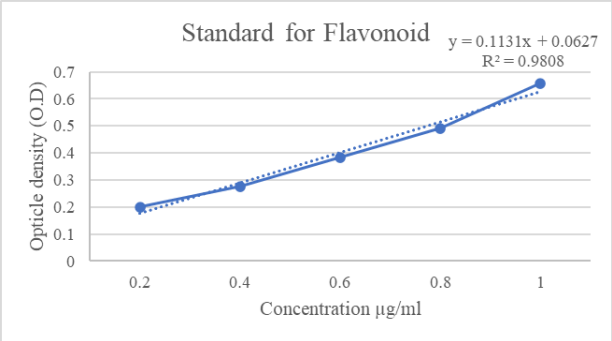


Figure:1: Standard graph for flavonoid

Total Phenolic Content:

The Folin-Ciocalteau method has used the concentration of the total phenolic. Table :1 shows total content of phenols and flavonoids.

In *B. lanzan* leaves methanolic (7.497mg GAE/g.) and hexane (0.898 mg GAE/g.) extract has higher phenolic compound as compared to leaves methanolic (1.964 mg GAE/g.) and hexane extract (0.857 mg GAE/g.). While in *M. peguenensis*, fruit methanolic (1.344 mg GAE/g.) and hexane (1.007 mg GAE/g) extract contains higher phenolic compounds as compared to leaves methanolic extract (0.897 mg GAE/g) & hexane extract (0.695 mg GAE/g.) and bark methanolic extract (1.002 mg GAE/g.) & hexane extract (0.985 mg GAE/g.). In *E. alsinoides*, methanolic (1.614 mg GAE/g.) and hexane extract (0.811 mg GAE/g.) of leaves contain a higher amount of phenolic compound as compared to bark methanolic (1.514 mg GAE/g.) & hexane (0.790 mg GAE/g.) and root methanolic (1.426 mg GAE/g.) & hexane extract (0.727 mg GAE/g.).

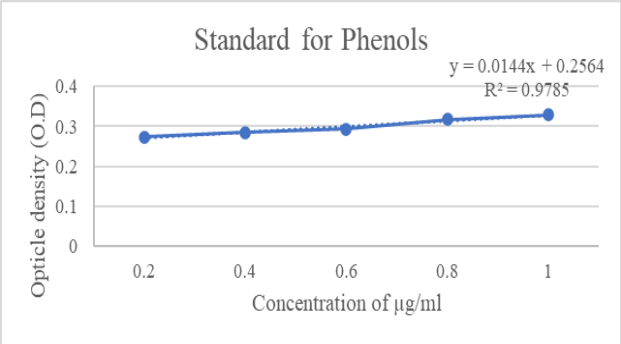


Figure:2: Standard graph for phenolics

DPPH Antioxidant Activity:

B. lanzan bark methanolic extract having highest ability to scavenge H^+ radicle and it's having least IC_{50} value 0.7162. *M. peguenensis* leaves have highest ability to scavenge H^+ radicle it's having least IC_{50} value 1.0508. *E. elsinoides* bark has highest ability to scavenge H^+ radicle it's having least IC_{50} value 0.6346.

Ferric reduction based FRAP assay

Buchanania lanzan methanolic extract of the bark has the highest Fe^{+2} scavenging activity (2.5271), while hexane extract of leaves (0.7803) had the least ability to reduce Fe^{+2} radicles. So here we observed that methanolic extracts of *B. lanzan* leaves and bark contain a higher reducing ability to scavenge Fe^{+2} radicles, but on the other hand nonpolar extraction of leaves contains the least reducing ability. In *Millettia peguenensis*, methanolic extract of Fruit (1.8556) has higher Ferrous ion scavenge activity while hexane extract of leaves (0.9778) has higher Ferrous ion scavenge activity. In *E. elsinoides* root methanolic extract (2.1639) has a higher ability to reduce the activity of Fe^{+2} radicles while hexane extract of leaves has a higher ability to reduce the activity of Fe^{+2} radicles In *Evolvulus alsinoides* methanolic and hexane extract of root contain a higher capacity to scavenge Fe^{+2} ions.

DISCUSSION

Methanol and hexane solvent systems have been used in this study to extract *B. lanzan* leaves, bark; *M. peguenensis* leaves, bark & fruits, and *E. elsinoides* leaves, bark & roots. Different phytochemicals will dissolve in methanol and hexane with a like dissolve like principle, so most polar compounds will dissolve more easily in methanol while most non-polar compounds will dissolve more easily in hexane. Studies proved that methanol has the highest polarity, so it will give us the highest extraction yield as

well as the presence of phenol, flavonoid, alkaloid, tannin, and terpenoids in the highest content.

Table:1 Total Phenolic Content and Flavonoid Content

SAMPLES	TPC		TFC	
	M	H	M	H
Buchanania lanzan				
Leaves	1.964±0.003	0.857± 0.001	7.497±0.347	4.225 ± 0.068
Bark	2.554±0.001	0.898±0.0009	7.336±0.021	15.556 ± 0.001
Millettia peguenensis				
Leaves	0.897±0.001	0.695± 0.002	10.262±0.044	5.165 ±0.021
Bark	1.002±0.002	0.985±0.002	5.249 ±0.021	4.170 ±0.028
Fruit	1.344±0.002	1.007±0.003	7.272 ± 0.013	6.318 ±0.034
Evolvulus alsinoides				
Leaves	1.614±0.001	0.811±0.002	5.411 ±0.036	5.975 ± 0.020
Bark	1.514±0.002	0.790±0.004	7.059 ± 0.021	4.401 ±0.021
Root	1.426±0.002	0.727±0.004	15.411 ± 0.013	7.082 ± 0.021

Here M is stands for methanolic extracts and H is stands for hexane extracts

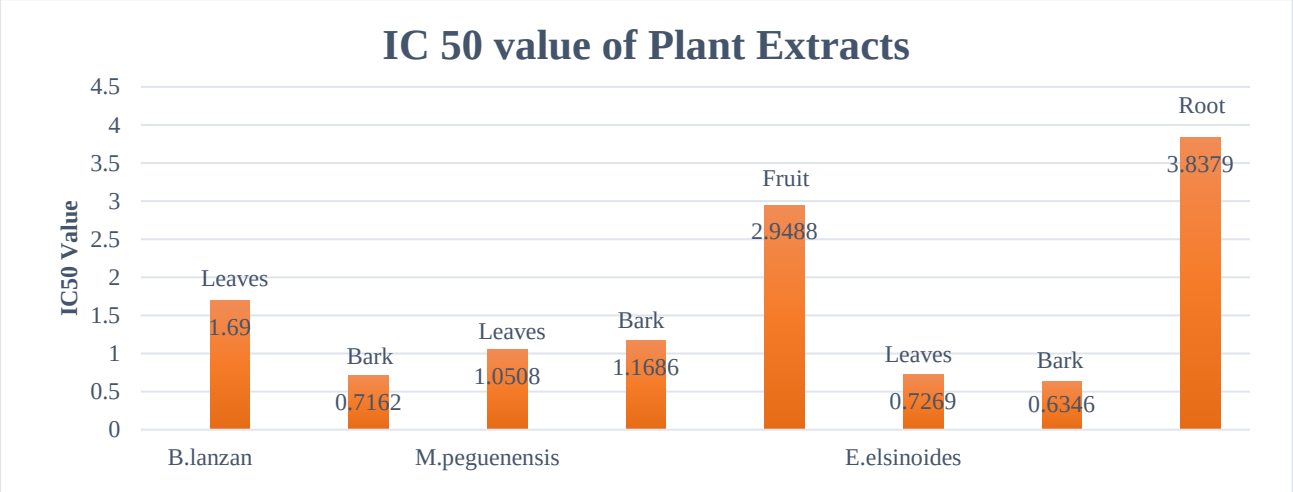


Figure:3: IC $_{50}$ Value of methanolic plant extract

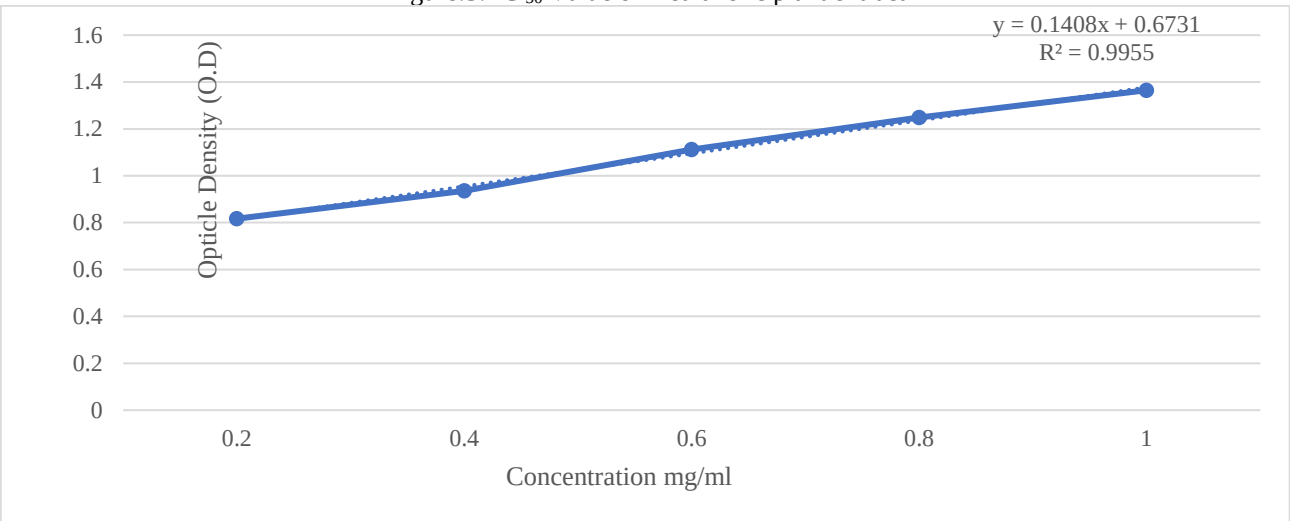


Figure:4: Standard graph for FRAP assay

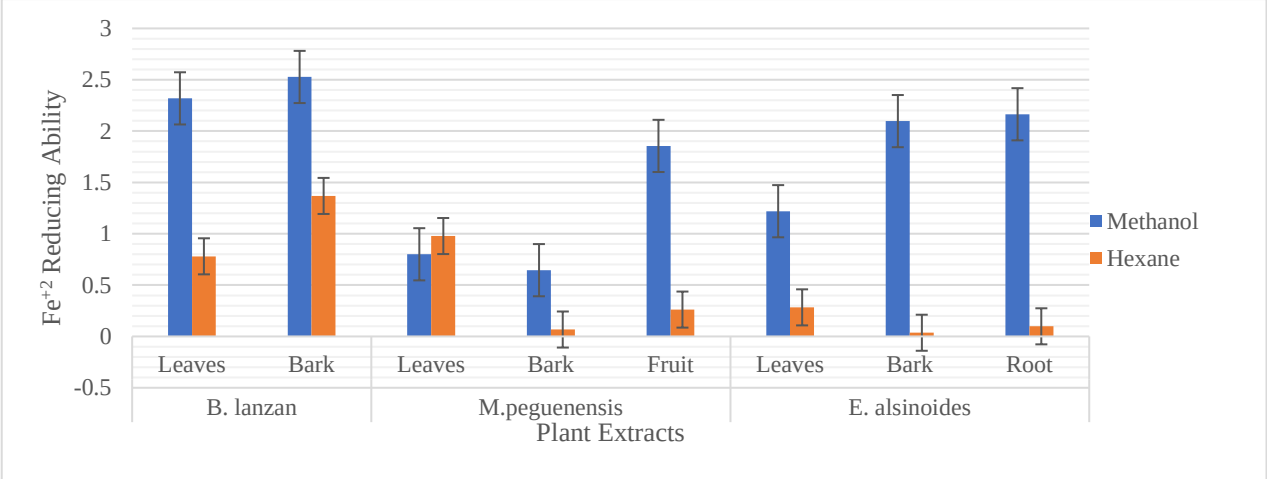


Figure:5 Reducing ability of Fe^{+2} radicles by Different plant parts in methanolic and hexane solvents

The highest rate of phenolics in extracts of *B. lanzan*, *M. peguenensis*, and *E. elsinoides* appears to be in the methanolic extracts. It is found that *B. lanzan* hexane extract of bark shows the highest levels of flavonoid. However, other plant methanolic extracts of *M. peguenensis* and *E. elsinoides* are the richest in flavonoids in comparison to hexane extracts.

A study showing that methanolic extracts of plant parts have the highest antioxidant and free radical scavenging capacity demonstrates that polyphenols such as phenols and flavonoids are the most important contributors. They possess strong antioxidant activity and act to defend the body from oxidative damage by scavenging radicle H^+ and Fe^{+2}.Interestingly, the *E elsinoides* root has the highest ability to scavenge H+ and the lowest IC_{50}; however, the methanolic extract of *B. lanzan* bark shows the highest absorbance, that's why it has the highest ability to reduce Fe+2 radicles.

In conclusion, these results suggest that these plant parts have potent antioxidant activity, so that these plant parts can be used for further drug development.In addition, research is underway in order to evaluate the plant's complete phytochemical and pharmacological profile to justify its traditional applications and reported antioxidant properties. A significant amount of phytochemicals suggest that these plants can be used to identify other biological and pharmacologic activities like anti-inflammatory, antimicrobial, anti-fungal, and many more. Further study on the plants should be done to unlock their potential.

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