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PHYTOCHEMICAL SCREENING AND ESTIMATION OF ANTIOXIDANT POTENTIAL OF SOME SELECTED MEDICINAL PLANTS FROM GWALIOR REGION

Biological Science

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ABSTRACT

Traditional medicinal still remains the first choice for primary treatment of diseases. The present study focuses on investigation of pharmaceutical properties of 5 selected plants namely *Psidium guajava, Moringa olifera, Ocimum sanctum,Citrus lemon, and Murraya koenigii* from Gwalior region. The crude was extracted in aqeous and petroleum ether for further testing. Phytochemical screening of the extract were evaluated. Antioxidant potential of plant extract were checked by NO, DPPH radical, H_2O_2 scavenging activity. All extract exhibited significant amount of secondary metabolites present in the aqeous and petroleum ether extract. The highest phenol content and flavanoid were observed in Psidium guajava for both extract. Compared to control all plants displayed significant antioxidant potential. The obtained activities of the tested extract could be due the presence of different terpenoid, alakaloid, flavonoid and others present in the plants.

KEYWORDS

Medicinal Plants, phytochemical screening, antioxidant, secondary metabolites

INTRODUCTION

In nature plants having certain chemicals which are known as phytochemicals. Recently photochemical have immense attention because they have various medicinal value [1]. Plant products have certain organic constitutes which have distinct physiological action on the human body and these active phyto ingradients like alkaloids, tannins, terpenoids, carbohydrates, flavonoids, and steroids. These active constitutes take part in an essential role against various ailments such as arthritis, allergy and cancer etc. Fortunately, these phytochemicals do not have any serious side effects like synthetic medicines. To develop healthy life for humans and animal's traditional folk treatment from wild plants has always guided researchers to search for novel medications [2]. Medicinal natural products of animal and plant origins was described by Hippocrates (ca. 460–377 BC), one of the ancient authors, who listed approximately 400 different plant species for medicinal purposes. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Ayurvedic, Chinese and Egyptian [3].

Over the years they have assumed a very necessary stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis according to World Health Organization. Free radicals, produced in the body by biochemical reactions, are causing factor in contributing to cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, diabetes, hair loss and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [4][5][6]. This paper mainly deals with collection, extraction, qualitative and quantitative analysis of phytochemicals.

MATERIALAND METHOD

Plant collection and identification

In this study, the plants were collected from campus of VRG College, Gwalior, Madhya Pradesh and its surroundings and they were authenticated by department of Botany, VRG College, Gwalior.

Preparation of plant extracts

The plant leaves were washed with tap water about 3-4 times. For evaporating the water content, the washed plants leaves were kept in for drying. After drying, for two weeks the plant leaves were air dried at room temperature (30^0C) and then grinded into uniform powder and different extracts were prepared using this powder. Then for the future use with proper labeling, the powder stored in air tight container [7].

Preparation of different extract using different solution of air dried powder of plants

The extract of leaf sample is prepared by using soxhlet apparatus. 10g of air dried powder in 100 ml of desire solution. This apparatus workon higher temperature with evaporation process. Extract preparationby soxhlet apparatus used for 12 hour continuously for each solution.The extracts are then filtered using filter paper.

Phytochemical analysis

To check the presence of plant primary and secondary metabolites such as carbohydrate, saponin, tannins, reducing sugar, flavonoids, glycosides and alkaloids Qualitative assay were carried out on the extract of the different plants leaves. The presence or absence of the phytochemical constituents of material was analyzed using the following standard methods [8].

Test for flavonoids

Few drops of ferric chloride solution added in test solution which would result in the formation of blackish red color shows the presence of flavonoids [9].

Test for carbohydrates

Benedict's test–few drops of Benedict's reagent was added in test solution (alkaline solution containing cupric citrate complex) and boiled in water bath, reddish brown precipitate was observed which shows a positive result for the presence of carbohydrate [10].

Test for Alkaloids

1ml aqueous extract was mixed and placed in 1% of aqueous hydrochloric acid on a steam bath, then, 1 ml of the filtrate was treated with Dragedorff's reagent. Presence of turbidity or precipitation shows the presence of alkaloids [11].

Test for Saponin

In a water bath 2 gm of powdered sample was boiled in 20 ml of distilled water and filtered. 10 ml of the filtrate was added in 5ml of distilled water and vigorously shaken for formation of stable froth. 3 drops of olive oil was mixed in frothing and vigorously shaken, then observed for the formation of emulsion [12].

Test for Phenols

0.5 ml of extract was added in 5 ml of Folin Ciocalteu reagent and 4ml of aqueous sodium carbonate. Blue color formation indicates the presence of phenols [7].

Test for Tannins

0.5 g of the extract and 10 ml of water was mixed and boiled in a test

tube and then filtered. A few drops of 0.1% ferric chloride was added and a blue-black color or brownish green shows the presence of tannins [13].

Test for Phlobatannins
Fruit extracts were boiled with 1% aqueous Hydrochloric acid. Formation of red precipitate indicates the presence of phlobatanins.

Test for Terpenoids
5 ml of each extracts were mixed in 2 ml of Chloroform and 3ml Concentrated sulphuric acid was carefully added to form a layer. A reddish brown colour at the interface indicates the presence of terpenoids [14].

Test for Cardiac glycosides
5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of Ferric chloride solution and then 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of a deoxysugar which is characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just spreading gradually throughout the layer [14].

Determination of total flavonoids content assay
About 10 g of the plant samples were extracted repeatedly with 100 ml of aqueous, petroleum ether, chloroform, methanol, ethanol, benzene (80% accept water) at room temperature. After that solution was filtered through Whatman filter. The filtrate was then transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weight [15].

Determination of total phenols content assay
Total phenols were determined by Folin Ciocalteu reagent. 0.5 ml of extract was added into 4 ml of aqueous Sodium carbonate and 5 ml of Folin Ciocalteu reagent. The Absorbance was read at 765 nm after 15 mins of incubation at room temperature. Gallic acid was used for preparing standard curve. Total phenols were expressed in terms of Gallic acid equivalents (mg CAE/100 g FW) [8].

Determination of nitric oxide scavenging activity assay
The spectrophotometry method was used to measure Nitric oxide scavenging activity [16]. Sodium nitroprusside (5mmol) in phosphate buffered saline was mixed with a control without the test compound. Test solutions at different concentrations (7.8-1000 µg/ml) were dissolved in methanol and incubated at 25° C for 30 min. 1.5 ml of the incubated solution was removed after 30 min and diluted with1.5 ml of Griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% Naphthyl ethylenediamine dihydrochloriode). At 546 nm the absorbance of the chromophores formed during the subsequent coupling with Naphthyl ethylenediamine dihydro chloriode was measured.

The below formula was used to calculate the inhibition of nitric oxide free radical in percentage:

Inhibition (%)=[(A control-A test)/A control] x 100

Where A control is the absorbance of the control (solution without extract) and A test is the absorbance of samples (extract and ascorbic acid).

Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity assay
DPPH assay was carried out as described by Hsu et al. [17]. With slightly modifications. 1.5 ml of 0.1 mM DPPH solution was added with1.5 ml of various concentrations (10 to 500 µg/ml) of leaf extract. The mixture was vigorously shaken and incubated in dark at room temperature for 30 min and reading the absorbance at 517 nm by a spectrophotometer is used to measure the reduction of the DPPH free radical. DPPH and methanol was used as control. The experiment was replicated in three independent assays. positive controls ascorbic acid was used. Inhibition of DPPH free radical in percentage was calculated by the formula:
Inhibition (%) = [(A control-A test)/A control] x 100

Where A control is the absorbance of the control (solution without extract) and A test is the absorbance of samples (extract and ascorbic acid).

Determination of Hydrogen Peroxide scavenging activity Assay
The ability of the plant extracts to scavenge hydrogen peroxide was determined according to the method of A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogenperoxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxideat 230 nm was determined 10 minutes later against blank solutioncontaining the phosphate buffer without hydrogen peroxide [18].

The percentage of hydrogen peroxide scavenging of both plant extracts and Ascorbic acid standard compounds werecalculated:
Inhibition (%) = [(A control-A test)/A control] x 100

Where Acontrol is the absorbance of the control (solution withoutextract) and Atest is the absorbance of samples (extract and ascorbicacid).

RESULT
From the qualitative analysis of leaves of selected medicinal plants, the presence or absence of tannins, alkaloids, saponin,, cardiac glycosides, flavonoids, terpenoids, phlobatamins and phenols was investigated. The results of this study are shown in the following Table 1 and 2. The result of these analyses of leaves of medicinal plants showsthat saponin, alkaloids, cardiac glycosides, phenols, Tannins, terpenoids, phlobatamins and flavonoidsare present in leaves of *Psidium guajava, Moringa olifera, Ocimum sanctum,Citrus lemon,* and *Murraya koenigii.*

Alkaloid, Saponin, Flavonoids, Steroids, Phenolic, Tannin, were present in water Extract weak presence in Petroleum ether.

Table-1 Phytochemical screening of crude aqueous extracts of *Psidium guajava, Moringa olifera, Ocimum sanctum,Citrus lemon, and Murraya koenigii*

Phytochemicals	Plant species				
	Psidium guajava	*Moringa olifera,*	*Ocimum sanctum*	*Citrus lemon,*	*Murraya koenigii.*
Saponin	+ + +	++	++	++	++
Flavonoids	+++	++	+++	++	++
Phlobatamins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Tannins	+	+	+	+	+
Cardiac glycosides	++	+	+	+	+
Phenols	+++	+++	++	++	++
Alkaloids	++	+	+++	+	+

Where +++ shows strong presence, ++ shows partially strong, + shows week and – shows absence of phytochemical activities.

Table-2 Phytochemical screening of crude petroleum ether extracts of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon, and Murraya koenigii.*

Phytochemicals	Plant species				
	Psidium guajava	*Moringa olifera,*	*Ocimum sanctum*	*Citrus lemon,*	*Murraya koenigii.*
Saponin	++	+	+	+	+
Flavonoids	++	++	++	++	+++
Phlobatamins	+	+	+	+	+
Terpenoids	++	+	++	++	+
Tannins	+	+	+	+	+
Cardiac glycosides	+	+	++	+	+
Phenols	++	++	++	++	++
Alkaloids	++	+	++	+	+

Where +++ shows strong presence, ++ shows partially strong, + shows week and – shows absence of phytochemical activities.

Determination of total phenolic content
Table 3 shows the total phenolic content of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii* in*petroleum ether*. It was observed from the table 3 that aquous extractcontains nearly more phenolic content then petroleum ether extract. These phytochemical protect the cells from oxidative damage

caused by free radicals.

Table-3 Total phenol content in the *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii* in aqueous extract.

Petroleum ether Leaf extract of plants	Total Phenol content (mg/g)
Psidium guajava	0.274±0.023
Moringa olifera	0.142±0.026
Ocimum sanctum	0.198±0.03
Citrus lemon	0.154±0.037
Murraya koenigii	0.132±0.028

Values are expressed as Mean ±SD for six different preparations

Table-4 Total phenol content in the *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon, and Murraya koenigii* in petroleum ether extract.

Aqueous extract of plants	Total Phenol content (mg/g)
Psidium guajava	0.212±0.012
Moringa olifera	0.117±0.009
Ocimum sanctum	0.113±0.041
Citrus lemon	0.116±0.019
Murraya koenigii	0.119±0.038

Values are expressed as Mean ±SD for six different preparations.

Determination of total flavonoid content

Total flavonoids content of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii*were shown in Table 5. Total maximum flavonoids content of *Psidium guajava*was found to be 0.191mg/g in aqueous extract. The contents of total flavonoids of different extract shown in Table 5 and 6

Table-5 Total flavonoid content in the *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii* in aqueous extract.

Aqueous extract Leaf extract of plants	Total Flavonoid content (mg/g)
Psidium guajava	0.191±0.032
Moringa olifera	0.172±0.096
Ocimum sanctum	0.138±0.081
Citrus lemon	0.114±0.023
Murraya koenigii	0.119±0.045

Values are expressed as Mean ±SD for six different preparations.

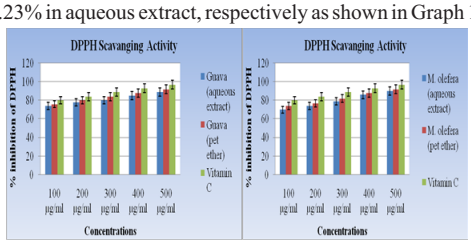
Table-6 Total flavonoid content in the *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii* inpetroleum ether extract.

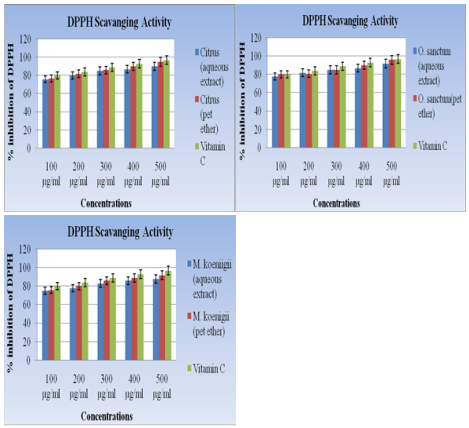
Petroleum ether Leaf extract of plants	Total Flavonoid content (mg/g)
Psidium guajava	0.178±0.064
Moringa olifera	0.159±0.021
Ocimum sanctum	0.121±0.096
Citrus lemon	0.113±0.042
Murraya koenigii	0.098±0.052

Values are expressed as Mean ±SD for six different preparations.

Determination of DPPH scavenging activity

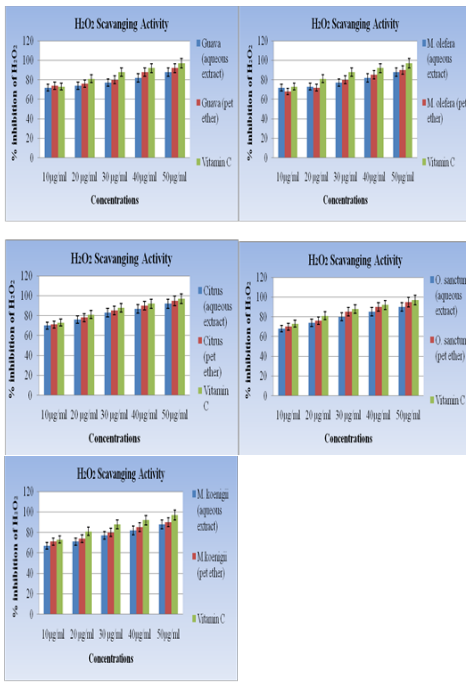
DPPH radical scavenging activity of dry leaf extract of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii*was compared with Ascorbic acid. At a concentration of 500(µg/ml), the scavenging activity of petroleum ether of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii* reached 92.33%, 93.12%, 96%,95.34% and 97.87% while at the same concentration; the standard was72.56%. At 500 µg/ml other solution extract found 97.23%, 96.37%, 90.12%, 92.22%, and 88.23% in aqueous extract, respectively as shown in Graph 1-5.





Determination of Hydrogen peroxide scavenging activity

Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon, and *Murraya koenigii* leaf extract scavenged hydrogen peroxide which may be attributed the presence of phenolic group. The scavenging of H_2O_2 was found to increase in dose dependent manner. Maximum inhibition of H_2O_2 was observed in the extracts of petroleum ether at highest concentration (600µg/ml) for the sample.), the scavenging activity of petroleum ether of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii*reached 97.33%, 93.12%, 96%,95.34% and 97.87%. At 500 µg/ml other solution extract found 92.23%, 90.37%, 92.72%, 95.72%, and 90.43% in aqueous extract, respectively as shown in Graph 6-10.



CONCLUSION

Recently, much attention has been directed toward plant extracts and biologicallyactive compounds isolated from popular plant species. The results exposed the occurrence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which established the well-known phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals supply medicinal as well as physiological properties to the plants studied in the treatment of different ailments. To conclude, the above experiments clearly indicate that different extract of dry leaf powder of *Psidium guajava, Moringa olifera, Ocimum sanctum, Citrus lemon,* and *Murraya koenigii* showed effective free radical scavenging activity which can be attributed to the presence of Flavonoids, and phenolics along with other compounds. Phenolic compounds, flavonoids and other secondary metabolites were detected in two different extract.

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