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PHYTOCHEMICAL SCREENING AND QUANTITATIVE ANALYSIS OF EXTRACT FROM AEGELE MARMELOS, CATHARANTHUS ROSEUS, GARCINIA PEDUNCULATA, MUSA PARADISIACA AND OCIMUM SANCTUM

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ABSTRACT

Ayurveda is one of the traditional medicinal systems of Indian culture. The philosophy behind Ayurveda is to prevent unnecessary sufferings and allow us to live a long healthy life. It involves the use of natural elements to eliminate the root cause of a disease by restoring balance between the three doshas (vata, pitta and kapha) within the body. Herbal medicines have existed world-wide since ancient period. World Health Organization (WHO) has estimated that 80% of the world's inhabitants still rely on traditional medicines for their health care. India is known to be one of the major biodiversity centers with about 45,000 plant species, including 15,000 medicinal plants. The concept of polyherbalism is to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plant are insufficient to achieve the desirable therapeutic effects. This study was conducted to screen the phytochemical constituents and to determine the levels of the major and trace elements of five medicinal plants used for the treatment of diabetes mellitus namely; Aegele marmelos, Catharanthus roseus, Garcinia pedunculata, Musa paradisiaca and Ocimum sanctum. The air dried leaves of the plants were subjected to soxhlet extraction using ethanol, petroleum ether, chloroform and aqueous. The crude extracts were obtained and subjected to screening for their phytochemical constituents such as alkaloids, tannins, terpenoids, reducing sugars, flavonoids, saponins, phenolic compounds and steroids using various standard methods and reagents. Trace metals in the five medicinal plants were analyzed quantitatively using Flame Atomic Absorption Spectroscopy. A digestion procedure involving the use of 4 ml of perchloric acid and 10 ml of aquaregia was performed to digest the medicinal plants. Sterols, tannins, terpenoids, flavonoids, alkaloids, saponins and reducing sugars were identified in the leaves of all the five plants. Elemental concentrations of some of the elements were obtained from the leafy materials in varying quantities. Ten heavy metals (Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Zn, and V), two

alkali metals (K and Na) and three alkaline earth metals (Ca, Mg and Al) and two halogens (Cl and Br) were quantitatively analysed. The anti-diabetic properties of the plant extracts could be attributed due to the presence of steroids, triterpenes and alkaloids. The concentration of toxic metals in these medicinal plants were found to follow the order Fe > Mn > Zn > Cu > Ni > V > Pb > Co > Cr > Cd. Sodium content was found to be very high in G. pedunculata while chlorine content was found to be very high in M. paradisiaca.

Keywords: Phytochemical Screening, Phytotherapy, Traditional Medicine, Polyherbal Formulation, Anti-diabetic Activity, Indigenous plants.

INTRODUCTION

Medicinal plants have been used for a long time since ancient period in the form of traditional medicine. Ethno-botanical information on medicinal plants have been used by indigenous cultures in the conservation of traditional medicine, biodiversity, community health care and drug development. The authentic knowledge of the usage of medicinal plants passed from one generation to another, after refining and addition. The folk recipes were prepared either from the whole plant or from their different parts, like stem, bark, root, flower, seed, etc. or from their secondary product such as gum, resins, and latex. In the human body, medicinal plants interact directly or indirectly with the body chemistry by their chemical constituents. Once the active constituents are absorbed into the blood, these constituents derive the required benefits by influencing or circulating the blood stream. Plants supply minerals, vitamins, and certain hormone precursors in addition to protein and energy to human body. Trace elements have significant roles in preventing a variety of human diseases and ailments, as observed by the study of elements with respect to indigenous medicinal plants.

Diabetes Mellitus is a common metabolic disorder, arising from a variety of pathogenic mechanisms, which result into hyperglycaemia. India is facing a diabetic explosion, according to the World Health Organization (WHO) estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030. The International Diabetes Federation (IDF) have reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025. The major sources of morbidity of diabetes are chronic complications that arise from prolonged hyperglycaemia, including retinopathy, neuropathy, nephropathy and cardiovascular disorders.

Diabetes is a disease of metabolism due to deficiency of insulin. Blood sugar level is maintained constant at a value of 70 to 120 mg of glucose/100 ml. Though several hormones are involved in the maintenance of diabetes, the most important ones are insulin and glucagon. Diabetes is caused as a result of loss balance effect of these hormones, usually due to less insulin production. Sugar starts to accumulate in the blood and blood sugar level increases and sugar passes into urine along with other minerals.

There are two types of diabetes. They are diabetes insipidus and diabetes mellitus. Diabetes mellitus is a condition in which a person's blood sugar level rises more than normal due to the deficiency of insulin or improper response to the insulin produced by the body cells. This disturbs metabolism of protein and other factors in the body. Diabetes mellitus is made up of two types: Type I and Type II.

Type I diabetes also referred to as juvenile diabetes, is insulin dependent and known to affect only 5% of the diabetic population. The Type II, which is non-insulin dependent, usually develops in adults over the age of 40. Type II diabetes often show no symptoms. It occurs as a result of the decline in cell membrane insulin sensitivity that can be aggravated by the consumption of high-glycemic carbohydrates, obesity, lack of exercise and aging process. At the moment a lot of orthodox drugs are in the system for the treatment of diabetes, especially the Type I, which is insulin-dependent. In our local communities, a lot of medicinal plant species are also currently used to manage/treat the Type II diabetes. Some of these medicinal plant species are Agele marmelos, Catharanthus roseus, Garcinia pedunculata, Musa paradisiaca and Ocimum sanctum among many others.

Aegele marmelos is commonly known as wood apple or Bael/Bilva, and belongs to Rutaceae family. This herb has great medicinal, spiritual and religious significance. Its fruits and leaves are considered sacred and used as offerings to the Hindu Gods like Lord Shiva. This is why it is also known as "Shiva druma" or the tree of Shiva in ancient scriptures. The roots, leaves, bark, fruits and seeds are used extensively in the Indian traditional system of medicine such as in Ayurveda and in various folk medicine to treat ailments. Although this plant is native to Northern India it is widely found throughout the Indian peninsula and in Burma, Ceylon, Indo-China and Thailand.

Bael fruits are of dietary use and the fruit pulp is used to prepare delicacies like puddings, murrabas and juice. They are also used in the treatment of dysentry, chronic diarrhea, and peptic ulcers, as a laxative and to recuperate from respiratory affections in various folk medicines. Leaves of this plant used to cause infertility/abortion in women.

Different organic extracts of the leaves of A. marmelos have been reported to possess alkaloids (halfordino, ethylcinnamamide, marmeline, Agelin, aegelenine, marmeline, dictamine, fragrine, O-methylhalfordinine, Oisopentanylhalford iniol, N-4-methoxy styryl cinnamide), cardiac glycosides, terpenoids (α-Phellandrene, α-Phellandrene, p-cymene, Limonene), saponins, tannins (skimmianine, Carotenoids, umbellliferone), Coumarin (Marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methylether, xanthotoxol, scoparone, scopoletin, umbelliferone, psoralen and marmelide), Polysaccharide (Galactose, arabinose, uronic acid and L-rhamnose), flavonoids and steroids. Aegle marmelos fruit pulp is reported for the availability of steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat and oil, inulin, proteins, carbohydrates, alkaloids, cardiac glycosides and flavonoids.

Figure 1: Aegele marmelos

Catharanthus roseus is an evergreen sub herb plant growing to 1m tall. The leaves are oval to oblong, 2.5-9.5 cm long, 1-3.5 cm broad, glossy green hairless with a pale midrib and a short petiole about 1-1.8 cm long. They are arranged in the opposite pairs. The flowers are white to dark pink with a dark red centre, with a basal tube about 2.5-3 cm long and a corolla about 2-5 cm in diameter with 5 petal like lobes. The fruits are a pair of follicles about 2-4 cm long and 3 mm broad.

The ethanolic extracts of the leaves and flower of Catharanthus roseus showed a reduction in blood sugar as compared to the standard drug glibenclamide. The hypoglycemic effect occurs due to the result of the increase in glucose utilization in the liver. The aqueous extract was found to lower the blood glucose level to about 20% in diabetic rats when compared to that of the dichloromethane and methanolic extracts which lowered the blood glucose level to around 49-58%. The hypoglycemic effect has occurred due to the result of the increased glucose utilization in the liver. The hypoglycaemic activity of alkaloids isolated from Catharanthus roseus have been studied pharmacologically and a remedy derived from the plant has been marketed under the proprietary name Vinculin as a treatment for diabetes

Figure 2: Catharanthus roseus

Garcinia pedunculata is an indigenous medicinal plant that belongs to the family Clusiaceae and is commonly known as "Taikor" in Bangladesh and "Amlavetasa" in India. It is especially available in North eastern states such as Assam and Arunachal Pradesh. The mature GP fruit is greenish yellow in color and is consumed as a raw fruit, or an ingredient for pickle preparation. It has been used locally for the ailments of asthma, bronchitis, cough, dysentery, fever and maldigestion. The fruit extract has been reported to possess a variety of beneficial effects such as antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, nephroprotective and cardioprotective activity.

GP fruit has been reported to contain β-carotene (45.00 mg/100 g), protein (0.50%), moisture (88.20%), riboflavin (0.02 mg/100 g) and thiamine (0.03 mg/100 g) as well as minerals (calcium 18.00, magnesium 23.00, manganese 0.23, sodium 1.80, potassium 106.00, iron 0.08, zinc 0.15, copper 0.12 and phosphorus 17.00 mg/100 g respectively.). Recently, the antioxidant constituent of the fruit was analyzed and was reported to contain phenolic (5.86 mg/g of catechins), flavonoids (5.60 mg/g of quercetin) and total anti-oxidant activity (504 nmol/g of ascorbate) as well as 6.67 mg of anthocyanins and 142.83 mg of ascorbic acid per 100 g, respectively. In addition, the presence of a number of benzophenones including pedunculol, garcinol and cambogin and other organic acids, such as citric acid, hydroxycitric acid, hydroxycitric acid lactone, and oxalic acid has also been identified. Further insights into GP's efficacy as free radical scavengers and capacity to protect cells from lipid peroxidation have been confirmed in several studies, thus uncovering its medicinal importance related to chronic or degenerative diseases including diabetes.

Figure 3: Garcinia pedunculata

Musa paradisiaca (Linn.) is a herbaceous plant measuring up to 9 m in length with a robust tree-like pseudo-stem, displaying a crown of large elongated oval deep-green leaves up to 365 cm in length and 61 cm in width. It belongs to the family Musaceae containing over 200 species; growing in the tropics and subtropics. It is seen as a major crop in Africa, Asia, and Central and South America consumed as energy yielding food;

estimated to provide about 60 million people in Africa with over 200 calories/day, contributing to their diet, nutrition and health. There are two commonly known names in the genus Musa (banana and plantain). For instance, plantain is the common name for herbaceous plants of the genus Musa and the fruits produced are used for cooking, in contrast to the soft, sweet banana and it is generally opined that there is no formal botanical distinction between bananas and plantains and that the use of either name (banana or plantain) is based mainly on how the fruits are consumed and that plantains are recognized to belong to the same species as banana. Analysis of ripe and unripe extracts of M. paradisiaca shows that it contains carbohydrate, protein, fat, fiber, ash and moisture. It is also found to provide calcium, potassium, manganese, sodium, zinc, phosphorus, nitrogen, iron and copper.

The fruit of M paradisiaca is used in the treatment and management of dysentery, diabetes mellitus, diarrhea, intestinal lesions, hypertension, ulcerative colitis . It acts as anti-microbial and anti-helmintic agents. The medicinal properties are believed to be associated with its constituents such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, terpenoids, alkaloids, and specific organic acids. Its leaves and seeds have been reported to possess wound healing property, anti-inflammatory, anti-oxidant and immune-promoting activities.

The use of unripe M. paradisiaca in the management of hepatic dysfunction and diabetes in STZ-induced diabetes in rats was assessed by Eleazu and Okafor. The authors reported that intake of unripe extract by diabetic rats resulted in a significant reduction in blood glucose when compared to diabetic control rats; urine glucose and protein decreased with time following treatment with extract of unripe M. paradisiaca. The authors indicated that the study shows the potential of unripe M. paradisiaca extract in the management of diabetes and diabetic complications.

Figure 4: Musa paradisiaca

Ocimum sanctum commonly known as holy basil or tulsi, is an aromatic perennial plant in the family Lamiaceae. It is native to the Indian subcontinent and widespread as

a cultivated plant throughout the Southeast Asian tropics. Tulsi is cultivated for religious and traditional medicine purposes and also for its essential oil. It is widely used as a herbal tea, commonly used in Ayurveda.

Holy basil is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems. Leaves are green or purple; they are simple, petioled, with an ovate blade up to 5 cm (2 in) long, which usually has a slightly toothed margin; they are strongly scented and have a decussate phyllotaxy. The purplish flowers are placed in close whorls on elongated racemes.

Tulsi leaves are known as elixir of life because of their diverse healing properties. From strengthening immunity, fighting bacterial and viral infections to warding off some of the most common ailments the benefits of tulsi plant are multifarious. This herb is said to improve pancreatic beta-cell function and insulin secretion, and further increases the uptake of glucose by muscle cells. Tulsi leaves are said to have hypoglycaemic properties, which lower blood sugar levels and help prevent complications of diabetes.

The genus Ocimum includes at least 30 species which have established in tropics and sub-tropical regions. Leaves and flowering tops are vital in extracting the essential oil. Oil of O. sanctum is discovered to have five fatty acids (stearic, palmitic,oleic and linoleic and linolenic acids). It is said to be resource of beta carotene, calcium, vitamin C. Eugenol, beta-cymne, Carvacol are naturally occurring chemicals in tulsi leaves which have tremendous use as anti-bacterial activity.

Figure 5: Ocimum sanctum

MATERIALS AND METHODS

EXTRACT PREPARATION

Fresh leaves and fruits of the plant species, Aegle marmelos, Catharanthus roseus, Garcinia pedunculata, Musa paradisiaca, and Ocimum sanctum (1 kg each) were collected from nearby town territories of Nalbari locale, Assam. The plant species were prepared in the form of voucher specimens and deposited in Assam Bio-resource Centre

Environment office in Katanipara, Assam. The leaves were washed and cut into pieces and air dried. The powdered plant materials were defatted using petroleum ether (60 to 80°C) using a Soxhlet extractor. The marc was further extracted by ethanol for 72 h to obtain the extract. The extract was filtered and evaporated to dryness under reduced pressure on a rotary evaporator. The concentrated extracts were dried by placing them in a dessicator. The weights of the crude extracts after drying were measured.

PHYTOCHEMICAL SCREENING

The crude extracts of the leaves of A. marmelos, C. roseus, M. paradisiaca, G. pedunculata and O. sanctum were subjected to phytochemical screening.

Test For Alkaloids

To a small amount of the dried extracts (free from ethanol), 5 ml of 10% HCl were added and stirred whiles heating. From the resulting mixture, 1 ml each of the filtrates pipetted into test tubes. Dragendorff, Mayer and Wagner reagents were added. In each case a sample test tube of each filtrate was reserved as reference.

Test For Saponins

To a small amount of the powdered samples, 2 ml of distilled water were added to each test tube and shaken vigorously. The formation of forth lasting for 15 min suggests the presence of saponins.

Test For Tannins

To 1 ml of dissolved extract, 2 ml of distilled water was added. Few drops of ferric chloride were added. The formation of blue black colour represents the presence of tannins.

Test For Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test For Phenolic Compounds

Extracts were added with few drops of ferric chloride solution. Formation of a bluish black coloration was observed. This confirms the presence of phenolic compounds**.**

Test For Phytosterols

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test For Reducing Sugars

Extracts were treated with Fehling's solution and heated. The formation of brick red precipitate indicates the presence of reducing sugar.

Test For Triterpenoid

Extracts were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Concentrated sulphuric acid was added latter. Formation of a brownish green ring at the contact of the two liquids indicates the presence of triterpenoid.

QUANTITATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT

Total Phenolic Content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent. The plant extracts (0.5 mL each) was mixed with 0.5 mL of FC reagent (1:1 diluted with distilled water) and incubated for 5 min at 22°C followed by addition of 2 mL of 20% Na_2CO_3. The mixture was then incubated further at 22°C for 90 mins and the absorbance was measured at 650 nm. The total phenolic content (mg/mL) was calculated using gallic acid as standard.

Total Flavonoid Content

The total flavonoid content (mg/mL) was determined using aluminium chloride ($AlCl_3$) method. The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was incubated for 5 mins at 25°C. This was followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. Two millilitres of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

SAMPLE PREPARATION FOR ATOMIC ABSORPTION SPECTROPHOTOMETRY

Plant samples were washed with deionised water and oven dried at 80°C for 2 days and then subjected to grinding for powder formation.

Digestion

Two gram powder of each plant sample (leaves) were dissolved in 10 ml of nitric acid for 12 h and then heated until the reddish brown fumes disappear. 4 ml of perchloric acid was added to the above solution and heated for 5 mins then 10 ml of aquaregia was added and heated to small volume and up to marked 250 ml by adding deionised water.

Atomic Absorption Spectrophotometry

Major and trace elemental contents were determined using flame atomic absorption spectroscopy using Perkin Elmer A Analysit 700.

Determination Of Elements

The dried alcoholic extracts were again subjected to flame atomic absorption spectrometry (AAS) to determine and quantify some basic elements in the leaves of the three plant species under investigation.

RESULTS AND DISCUSSION

Extractive Value

Plant Materials	Solvents			
	Water	Ethanol	Chloroform	Pet. Ether (40-60%)
A.G	22.68	15.84	6.41	2.24
C.R	23.21	12.22	4.65	2.88
G.P	26.48	14.10	3.78	2.21
M.P	19.15	10.26	2.86	1.58
O.S	24.20	13.18	5.73	2.50

Table 1: Extractive Value of Solvents

The present study was carried out on the five medicinal plant species, which revealed the presence of bio-active constituents of medicinal value. The phytochemical compounds of these five plants were qualitatively analysed and the results of the ethanol extracts of all the five plants were almost the same (Table 1). The phytochemical analysis showed good result for all major phytoconstituents. The qualitative analysis revealed the presence of the biomolecules such as alkaloids, anthraquinone, steroids, reducing sugars, coumarins, flavonoids, terpenoids, tannins and saponins in all the three medicinal plant species. The presence of these phytoconstituents may contribute to the pharmacological actions of these plants**.**

According to the literature, the pharmacological activity and the phytochemical compositions confirm the traditional use of some anti-diabetic plants. It is noted that the anti-diabetic effect results from several chemical elements: alkaloids, sterols, essential oils and triterpenes. The results of the phytochemical investigations on the plants under study confirmed the traditional use of these anti-diabetic plants. The pharmacology of these three plants can therefore be linked to the presence of alkaloids, triterpenoid and sterols phytoconstituents in the plants (Table 3).

The study also focused to estimate the concentrations of some toxic and essential metal ions in the plants. Ten heavy metals (Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Zn, and V), two alkali metals (K and Na) and three alkaline earth metals (Ca, Mg and Al) and two halogens (Cl and Br) were chosen on the basis of their importance to health.

The investigation revealed the presence of these elements in varying concentrations in the extracts as presented in Table 2. The accumulation of the elements varied from one plant organ to the other. Although, a lot of toxic/trace elements were recorded in the plants, these levels or concentrations were far below the World's Permissible Levels as indicated by Bowen (1979); Kloke (1979); Kabata-Pendais and Pendais (1984), thus, making the plant species, L. speciosa, B. ferruginea and M. alba safe for the production of herbal medicines, and in the management of Type II diabetes. Again, the high levels of iron (Fe) in the plant organs may probably make it a very powerful tonic to boost anaemic conditions in humans. In this regard M. alba with the highest Fe concentration presents itself as the best choice. It is worth mentioning that almost all the essential elements required for the natural remedies for diabetes are present in all the three plant species even though in lower quantities compared to the permissible levels. These are zinc (15 to 25 mg), which lowers blood sugar; chromium (200 to 1,000 mcg), improves glucose tolerance and magnesium (1,000 mg), that leads to improved insulin production in elderly people and reduces eye damage. The presence of these nutrients and the phytoconstituents in all the five plants makes these plants unique and significant good sources of natural remedies for diabetes.

There are no guidelines to establish a permissible level of metals in herbs. By monitoring the level of metals in medicinal plants one is able to indicate the level of environmental pollution in that area. Even though the plant samples were collected from locations exposed to some level of vehicular activities, the results does not point at any serious pollution concern in the area as at the time of collection.

The efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents like essential oils, vitamins, glycosides, etc. However, it has been established that over dose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different elements which causes various health problems. This is because these essential metals can also produce toxic effects when the metal intake is in high concentrations, whereas non-essential metals are toxic even in very low concentrations for human health (Sharma et al., 2009). Elemental contents of the medicinal plants are therefore very important and need to be screened for their quality control (Liang et al., 2004; Arceusz et al., 2010).

Phytochemicals	Agele marmelos			
	Chloroform	Ethanol	Petroleum Ether	Aqueous
Alkaloids	+	+	+	-
Saponins	-	+	-	+
Tannins	+	+	-	+
Flavonoids	+	+	+	_
Phenolic Compounds	-	+	+	-
Phytosterols	_	+	+	_
Reducing Sugar	_	+	+	_
Triterpenoids	+	+	_	+

Table 2: Phytochemical Analysis Test Chart of Aegele marmelos

(+) --- Positive

(-) --- Negative

Phytochemicals	Catharanthus roseus			
	Chloroform	Ethanol	Petroleum Ether	Aqueous
Alkaloids	+	-	_	+
Saponins	_	+	+	_
Tannins	+	_	+	_
Flavonoids	+	+	_	+
Phenolic compounds	_	_	+	+
Phytosterols	+	_	+	_
Reducing Sugars	_	+	_	+

Triterpenoids	+	_	+	_

Table 3: Phytochemical Analysis Test Chart of Catharanthus roseus

(+) --- Positive

Phytochemical Test	**Garcinia pedunculata**			
	Chloroform	**Ethanol**	**Petroleum Ether**	**Aqueous**
Alkaloids	+	-	+	-
Saponins	-	+	+	+
Tannins	_	+	-	-
Flavonoids	+	-	+	_
Phenolic Compounds	+	-	+	-
Phytosterols	-	+	+	+
Reducing Sugars	+	+	_	+
Triterpenoids	_	+	-	-

(-) --- Negative

Table 4: Phytochemical Analysis Test Chart of Garcinia pedunculata

(+) --- Positive

(-) --- Negative

Phytochemical Test	**Musa paradisiaca**			
	Chloroform	**Ethanol**	**Petroleum Ether**	**Aqueous**
Alkaloids	-	-	-	+
Saponins	+	-	-	+

Tannins	+	+	-	-
Flavonoids	+	+	-	+
Phenolic Compounds	+	-	+	+
Phytosterols	+	-	+	+
Reducing Sugars	_	+	-	+
Triterpenoids	+	-	+	-

Table 5: Phytochemical Analysis Test Chart of Musa paradisiacal

(+) --- Positive

(-) --- Negative

Phytochemical Test	**Ocimum sanctum**			
	Chloroform	**Methanol**	**Petroleum Ether**	**Aqueous**
Alkaliods	+	+	-	-
Saponins	+	-	-	+
Tannins	+	-	-	+
Flavonoids	+	+	-	+
Phenolic Compounds	+	+	+	+
Phytosterols	+	-	+	-
Reducing Sugars	-	+	+	+
Triterpenoids	+	+	-	+

Table 6: Phytochemical Analysis Test Chart of Ocimum sanctum

(+) --- Positive

(-) --- Negative

Element	AM	CR	GP	MP	OS
Br	93.94 ± 3.28	95.91 ± 4.83	1.24 ± 0.02	34.2 ± 2.83	87.08±3.23
Na	1658.90 ± 3.71	237.61 ± 1.40	160.65 ± 1.45	156.90 ± 2.70	30.2 ± 2.80
K	1.08 ± 0.03	1.38 ± 0.03	0.94 ± 0.02	1.10 ± 0.09	1.6 ± 2.70
Mg	0.07 ± 0.002	0.39 ± 0.01	0.04 ± 0.001	0.05 ± 0.001	1.90 ± 0.09
Cu	2.58 ± 0.03	4.57 ± 0.04	1.61 ± 0.01	2.34 ± 0.04	0.06 ± 0.001
V	ND	ND	0.40 ± 0.02	ND	2.30 ± 0.04
Cl	231.53 ± 2.54	1514.03 ± 10.81	ND	167.89 ± 3.45	ND
Al	7.61 ± 0.56	8.83 ± 0.55	169.40 ± 0.51	80.79±0.78	167.80 ± 0.34
Mn	6.58 ± 0.45	21.50 ± 0.55	3.95 ± 0.07	15.67±0.08	18.75±0.75
Ca	0.15 ± 0.003	0.11 ± 0.001	0.02 ± 0.001	0.18±0.001	1.5±0.05
Fe	22.14± 0.55	21.54± 0.55	47.90± 2.23	27.80±0.88	18.90±0.001
Zn	6.66± 0.47	4.10± 0.14	20.08± 0. 52	15.08±0.68	27.90±0.88
Pb	< 0.01± 0.001	< 0.01± 0.001	< 0.01± 0.001	<0.01±0.001	<0.01±0.001
Cr	<.006± 0.001	<.006± 0.001	<0.006± 0.0.001	<0.006±0.001	<0.006±0.001
Ni	1.34± 0.03	1.96± 0.04	0.34± 0.01	1.56±0.02	1.56±0.02
Cd	<0.002± 0.001	<0.002± 0.001	<0.002± 0.001	<0.003±0.001	<0.003±0.001
Co	0.015± 0.001	<0.005± 10.81	0.007± 0.001	0.014±0,001	0.014±0,001

Table 7: Basic Elements Obtained From The Leaves Of The Plant Species Using Atomic Absorption Spectrometry

Plant Name	Family	Part Investigated	Total Phenolic (mg) (GAE/100g)	Total Flavonoids (mg) (QE/100g)	Flavonoid /Phenolic (F/P ratio)
Aegele	**Rutacea**	**Leaves, Bark**	**65.20**	**11.37**	**0.17**

marmelos					
Catharanthus roseus	**Apocyanaceae**	**Leaves, Flower**	**42.8**	**5.24**	**0.12**
Garcinia pedunculata	**Clusiaceae**	**Fruit**	**80.14**	**23.38**	**0.29**
Musa paradisiaca	**Musaceae**	**Fruit**	**136.8**	**34.16**	**0.24**
Ocimum sanctum	**Lamiaceae**	**Leaves**	**16.5**	**4.49**	**0.27**

Table 8: Total Phenolic And Total Flavonoid Content

CONCLUSION

The objective of the studies were successfully conclude the extracts of aerial parts of Aegele marmelos, Catharanthus roseus, Garcinia pedunculata, Musa paradisiacal and Ocimum sanctum showed the presence of wide range of phytochemicals. Among five plants, significant number of phytochemicals principally passed in Garcinia pedunculata, Musa paradisiacal and Ocimum sanctum which revealed about the medicinal importance. Medicinal value was also justified in terms of phenolic and flavanoids content value which found as tremendous in Garcinia pedunculata and Musa paradisiaca. Screening of these five plants suggested that finest 03 plants based on phytochemical tests and content estimation may have anti-diabetic potential. As flavanoids and phenolic compounds have diverse activity profile, therefore, according to the higher content, their extracts could be useful for the management of Type-2 diabetes mellitus with minimal adverse drug events over the synthetic drugs. These investigations indicates towards the possibility of pure active principle constituents of natural origin from the extract with high potency which could lead to the isolation of novel natural compounds from screened plants.

FUTURE PROSPECTS

The Herbal formulations have its own importance and advantages as compare to any other forms of medicines. As discussed in the present research the herbal formulations are free from any undesirable side effects and more or less they are non habit forming. The Indian climate favours the growth of many rare varieties of medicinal plants. But

the need of the hour is, these plants should be identified and much extensive research should be done on it so that new Drug discovery can be made to cure many infectious diseases. Many research organizations and Industries are pursuing research on exploring the flora like Himalayan Drugs, CIMAP etc. and many success stories are daily published. But the research should be carried out in a large scale and should be region specific so that new formulations can be prepared. Much work is also going on Polyherbal Formulation, in which many herbal drugs are scientifically mixed to get the synergistic effect.

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