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Phytochemical Screening and Free Radical Scavenging Potential of Maha Vallathy Leghiyam Aqueous Extract

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Abstract
Phytochemicals such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils are a large group of plant-derived compounds commonly found in diets high in fruits, vegetables, beans and cereals. The sample of Maha Vallathy Leghiyam **(**MVL) was analyzed for their phytochemical compositions, vitamins and minerals constituents. Based on the observed results explored the presence of bioactive constituents comprising flavonoids, phenols. These substances are the main reason for beneficial role against human health related problems, and it is responsible for the anti-inflammatory, antioxidant, antimicrobial, antitumor and anticancer properties. During the present study, we have tested the Antioxidant activity of through DPPH scavenging assay. The phytochemical screening revealed the extract richness in Tannins, Phlobatannin, Saponins, Flavonoids, Steroids and Alkaloids. Antioxidant activity was determined by DPPH radical scavenging assay, in which IC_{50} values obtained by DPPH activity for MVL aqueous crude extract was found to be 50µg/mL. The obtained results suggest that MVL has promising antioxidant activity and could serve as potential source of natural antioxidants.

Keywords: Phytochemical; metabolites; alkaloids; minerals; antimicrobial property; MVL

Introduction

Phytochemicals are the plant chemicals obtained from various fruits, vegetables, nuts, pulses etc., which have been proved to reduce the risk of many chronic diseases[1-3]. These phytochemicals are classified into phenolics, terpenoids and steroids, alkaloids and other chemicals[4,5]. These are extensively used as anti-oxidants, anti-cancerous[6], cytotoxicants, anti-microbials, detoxifying agents, anti-rheumatics, anti-malarial, hepaticidal [78,9]etc. The discovery of antibiotics from plants has brought about a revolution in the medicine world as they are being used for treating various bacterial diseases[10,11]. As the microbes grew resistant to many of the existing antimicrobials, various plant derivatives have been reported for their studies involving active phytochemicals and bioactive compounds because of their replenished antibacterial activity. Due to the presence of synergistic effect, a plants crude extract is more liable for eliminating the microbes than the isolated components of the plant[12-15].

MahaVallathyLeghiyam (MVL), is one such phytochemical which is a crude mixture of plant extract. It is to be specific, a siddha herbal medicine which has an approximate of around 30-40 ingredients of biological importance[16]. It has been, in previous reports, prescribed as a drug for various ailments including as an anti-AIDS drug[17]. Many reports have demonstrated TLC and HPTLC studies of MVL proving its medical importance as an herbal drug. Also various drugs and its components are identified within herbal plants that are effective antibiotics (Basile et al., 2000)[18]. This study focuses on the anti-bacterial activity of MVL based on its constituents of phytochemical, vitamin, mineral and several other bioactive compounds concentrations. Since, medieval times Since, medieval times MVL has been in practice and prescribed for various ailments but its prognostic study over oral cancer is mere. Instead of surgical removal, chemotherapeutics and radiotherapy as a routine modality of treating this type of cancer, natural therapies can be a good substitute. Even this handmade preparation of MVL is rich in phytochemicals and antioxidants which can act a natural chemo preventant to inhibit oxidation associated free radicals.

MATERIALS AND METHODS

Flavonoids

From the given sample 2mL of extract was taken and mixed with few drops of 20% NaOH. Formation of intense yellow color was observed. To that yellow color mixture few drops of 70% diluted HCL was added. The disappearance of yellow color was observed. The formation and disappearance of yellow color indicate the presence of flavonoids.

Alkaloids

10ml of extract was taken from the given sample. In that, 8mL Picric acid was added. The formation of orange color was observed. And then the appearance of dark orange or purple color was observed. These color changes indicate the presence of alkaloids.

Saponins

From the given sample 2ml extract was taken and 5ml of distilled water was added, which is need to be vigorously shaken. The formation of bubbles and persistent form foam indicates the presence of saponins.

Tannin

2mL of extract was taken. In that 10% of alcoholic Ferric Chloride was added. The formation of brownish blue or black color was observed. The color change indicates the presence of tannin.

Phenolic Compound

2ml of extract was taken. In that 2ml of 5% Ferric Chloride was added. The formation of Blue color was observed. This indicates the presence of phenolic compound.

Terpenoids

From that given sample 1ml of extract was taken. In that 0.5ml chloroform followed by few drops of con. H_2SO_4 was added. The formation of reddish-brown color was observed. This indicates the presence of terpenoids.

Glycosides

1mL of given sample was taken. In that 0.5ml of glacial acetic acid and 1% aqueous Ferric Chloride were added. The formation of brownish ring was observed. This indicates the presence of glycosides.

Calcium ions

Add 10 drops of sample and few drops of aqueous Ammonia solution, followed by few drops of 1M Ammonium Oxalate. The formation of white precipitate indicates the presence.

Sulphate ions

Add 10 drops of sample, few drops of dilute HCl and again few drops of 1M barium chloride. Appearance of white precipitate indicates presence of Sulphate ions.

Carbonate

Add 10 drops of sample, few drops of concentrated HCl. Evolution of CO_2 indicates the presence of carbonate.

Starch

To 10 drops of sample, add few drops of 1M Iodine solution. Formation of blue black color indicates the presence of starch.

Reducing sugar

To 3mL drops of sample add Fehling's solution and Fehling's solution B. Kept in water bath for 15min. The formation of green suspension and red precipitate indicates the presence of reducing sugar.

Amino acid

Add 1mL of sample, 2or 3 drops of 0.5% Ninhydrin reagent. Kept in water bath for few min. Formation of purple/blue colour indicates the presence of amino acids.

Ferrous ion

Add 10ml of sample, few drops of 0.5 M Potassium Thiocyanate. Formation of deep red solution indicates the presence of ferrous ion.

Halides

To few drops of sample, add dilute Nitric acid and few drops of Silver Nitrate.
Formation of white precipitate indicates for chloride
Formation of cream precipitate indicates for bromide
Formation of yellow precipitate indicates for iodide

Phosphate

To few drops of sample add few drops 1M of Ammonium Molybdate. Formation of deep yellow color indicates presence of phosphate.

Sterols

To 2ml of solution, add 2ml of Chloroform, 2ml of concentrated Sulphuric acid. Chloroform layer appears red and acid layer shows fluorescence.

***In vitro* Antioxidant activity**

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging method was used to evaluate the antioxidant property of the sample. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the MVL extracts required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each sample was expressed in terms of IC50, and was calculated from the graph after plotting inhibition percentage against extract concentration DPPH assay was carried out after making some modifications in the standard protocol (28376770). 1.5 mL of 0.1 mM DPPH solution was mixed with 1.5 mL of various concentrations (10 to 500 µg/mL) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays.

Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula: Inhibition (%) = [(A control-A test)/A control] × 100

Where a control is the absorbance of the control (L-Ascorbic acid) and A test is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

RESULTS

Qualitative analysis of the Maha Vallathy Leghiyam (MVL)

The result of the preliminary phytochemical screening was carried out on the Maha Vallathy Leghiyam extracts of all the samples and revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids supporting the reason for its wide range of biological activities as showed in table 1. Tannins, Phlobatannins, Saponins, Flavonoids, Steroids and alkaloids were found to be universally present in crude extract of MVL.

Table 1. Qualitative analysis of the MVL

Flavonoids	Alkaloids	Saponins	Tannins	Phenolic compounds	Terpeoids	Glycosides	Sterols	Carbonate ion	Reducing sugar	Starch
++	-	-	+	+	+	+	+	-	+	+

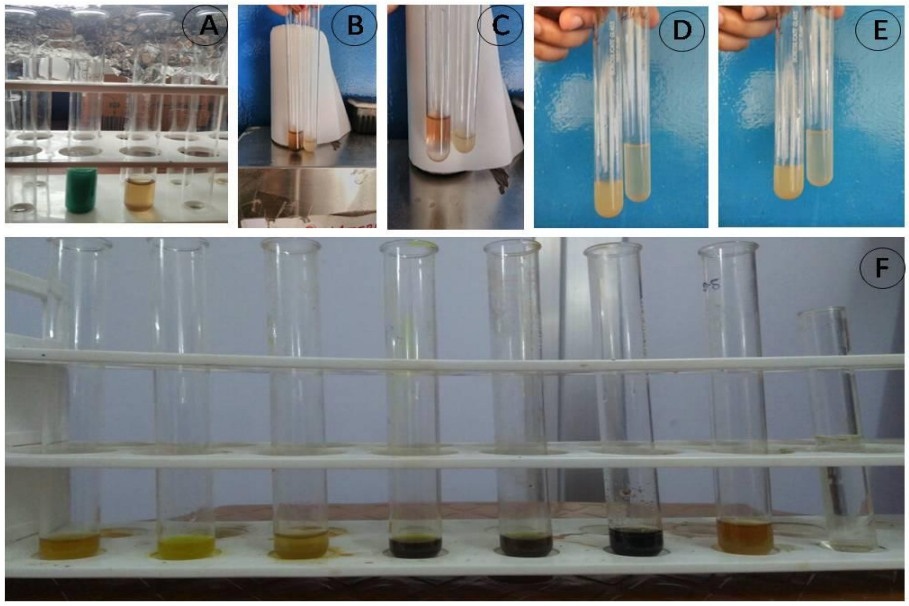

Figure 1: A –flavanoids and alkaloids, B-saponins, C-tannins, D-phenols, E-terpenoids, F-glycosides

Table 2: Solubility Analysis of MVL

Solvents	Hexane	Toluene	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water	DMSO
Lehiyam	+	-	++	-	-	++	+++	+++	+++

+++ Completely Soluble, ++ Moderately Soluble, + Barely Soluble with small particles, - indicates completely insoluble.

Qualitative analysis of MVL

FLAVONOIDS:

From the given sample 2ml of extract was taken and mixed with few drops of 20% NAOH. Formation of

intense yellow color was observed. To that yellow color mixture few drops of 70% diluted HCL was added. The disappearance of yellow color was observed. The formation and disappearance of yellow color indicate the presence of flavonoids.

ALKALOIDS:

10mL of extract was taken from the given sample. In that, 8mL Picric acid was added. The formation of orange color was observed. And then the appearance of dark orange or purple color was observed. This color change indicates the presence of alkaloids.

SAPONINS:

From the given sample 2 mL extract was taken and 5ml of distilled water was added, which is need to be vigorously shaken. The formation of bubbles and persistent form foam indicates the presence of saponins.

TANNIN:

2mL of extract was taken. In that 10% of Alcoholic Ferric Chloride was added. The formation of brownish blue or black color was observed. The color change indicates the presence of tannin.

PHENOLIC COMPOUND:

2 mL of extract was taken. In that 2mL of 5% Ferric Chloride was added. The formation of Blue color was observed. This indicates the presence of phenolic compound.

TERPENOIDS:

From that given sample 1 mL of extract was taken. In that 0.5ml chloroform followed by few drops of concentrated H_2SO_4 was added. The formation of reddish-brown color was observed. This indicates the presence of terpenoids.

GLYCOSIDES:

1mL of given sample was taken. In that 0.5mL of glacial acetic acid and 1% aqueous ferric chloride was added. The formation of brownish ring was observed. This indicates the presence of glycosides.

STEROIDS:

2mL of given sample was taken. In that 2 mL of Chloroform and 2mL Sulphuric acid was added. The formation of red color was observed in chloroform layer and fluorescence was observed in the acid layer

Table 3: Qualitative Analysis of the Compound in terms of Solubility

Sample	Flavonoids	Alkaloids	Saponins	Tannin	Phenolic compound	Terpenoid	Glycosides	Steroid
I. Methanol	++	+	+	-	-	-	-	+
II. Ethanol	-	-	++	-	-	-	-	-
III. Chloroform	-	+	+	-	-	-	-	-
IV. Hexane	-	-	-	-	-	-	-	-
V. Water	+	-	-	+	+	+	+	+
VI. DMSO	+	+	-	+	+	+	+	-

+++ present in high concentration, ++ present in medium concentration, + present in low amount, - indicates absence.

DPPH Radical Scavenging Activity

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. DPPH radical scavenging activities of the MVL extracts depended are the only on plant type but also upon the extraction of various formulations like siddha and medicinal plant extracts. In general, DPPH scavenging activities increased with water solvents of MVL. These phenolic components possess many hydroxyl groups including o-dihydroxy group which have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidant was able to reduce the stable radical

DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecules a whole. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in methanol solution centered at 517 nm. The dose response curve of DPPH radical scavenging activity of crude extracts of plant was observed, when compared with standard ascorbic acid and shown in figure 2. Antioxidant activity in the form of IC_{50} values of different extracts were calculated and shown in table 3. Highest antioxidant activity was given by MVL extract at the concentration of 50µg/mL among all the solvents.

Table 4: DPPH Radical Scavenging Activity – Antioxidant effect (%)

S.NO	SAMPLE µg/mL	Water	DMSO	ETHANOL	METHANOL
1	10	38.42±1.12	3.23±0.09	13.80±0.12	0.44±1.01
2	50	49.51±0.89	4.24±0.04	15.06±0.09	8.22±1.09
3	125	62.07±1.01	6.38±0.01	15.64±0.14	25.34±1.11
4	250	75.97±1.22	10.68±0.54	17.06±0.18	10.87±1.12
5	500	77.15±1.12	14.01±0.74	18.02±0.19	35.78±1.23
6	1000	81.56±0.98	19.72±0.81	24.61±0.19	40.36±1.02

Results shows *in vitro* antioxidant effect of different solvent mediated MVL extract. In this, aqueous solvent shows efficient antioxidant activity. The IC_{50} of the MVL has found to be 50 µg/mL, which shows 50% inhibitory effect.

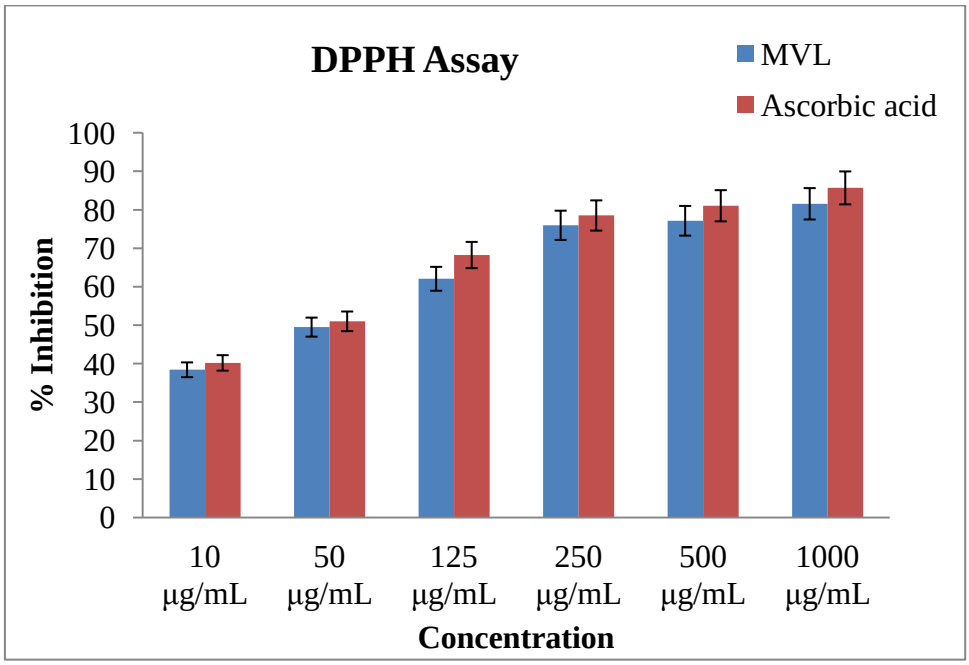

Figure 2: Free radical scavenging activity of MVL compared with standard Ascorbic acid

DISCUSSION

Natural compounds of herbal extracts have gained extensive importance due to their effective nature on various ailments. Their biological parameters as clinical products were well reviewed by Prof. (Dr.) CiddiVeeresham in the journal of advanced pharmaceutical technology and research [19]. A detailed explanation of secondary metabolites from natural extracts as a source of potential drug leads was reported by Daniel A Dias [20]. In his review he has reported these herbal extracts to be potential forms of primary and secondary metabolites which gave an insight to carryout this study. In this study, initially when the compound MVL was checked for a qualitative analysis, the traces of flavonoids, tannins, phenolic compounds, terpenoids, glycosides, sterols, reducing sugar and starch. Initially the flavonoids, sterols and tannins were detected by the disappearance of the yellow color by addition of diluted HCl proving MVL has anti-inflammatory activity based on previous report by Kim HP [21]. The traces of phenolic compounds, glycosides and the rest has proven the ability of MVL as an antioxidant and majorly as a potent anti-bacterial compound based on the studies of Nazeh M, Christina[22,23]. When the solubility test was done for MVL, hexane had minimal solubility while chloroform and ethanol showed moderate solubility and toluene, acetone and ethyl acetate were completely insoluble[24]. Methanol, water and DMSO had the maximum solubility and hence proving high polarity[25]. The DPPH assay showed that at a concentration of 1000 µg/mL, the compound MVL has shown maximum antioxidant [26] percentage of 81.56 in water as solvent. This was in correlation with the study of Ashafa who has reported an extensive work of antioxidant activity of herbal extracts. Thus, this study concludes that MVL has shown maximum rates of antioxidative, anti-inflammative and anti-bacterial nature because of the components present in it.

CONCLUSION

The finding of this study suggests that this MVL extract could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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Conflict of Interest

The authors declare no conflict of interest.

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