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Preliminary Phytochemical Analysis of *Amarathus viridis.*

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ABSTRACT

Phytochemistry or plant chemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structured of these substances, their biosynthesis, turnover and metabolism, their natural distribution and biological function. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other hand. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary and secondary metabolites. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances. Therefore, it is of interest to investigate the preliminary phytochemical analysis of *Amarathus viridis*

Keywords: *Amaranthus viridis* , Phytochemistry ,Secondary metabolites , Butanol ,Chloroform .

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INTRODUCTION

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds [1] Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem [2] Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [3,4].

They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [5]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of them may result in the discovery of novel active compounds [6] Despite of tremendous progress in human health care system, the infectious diseases caused by microorganismsare still a major threat to the public health [7].

Plants contain hundreds or thousands of metabolites. Medicinal and aromatic plants, a gift of the nature, are being used against various infectious diseases in the world since the past history. The discovery, development and the use of modern medicines have a deep routed connection with the age old practice of folk and traditional medicinal background of the natives. Thus the ancient wisdom has been the basis of modern medicine and therapeutics [8].

Natural antimicrobials have been often derived from plants, animal tissues or microorganisms. The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants [9,10] Medicinal herbs constitute effective sources of flavonoids, tannins, glycosides, anthraquinones, steroids antimicrobial and antioxidant natural products [11] and terpenoids. They do not only protect the plants but Medicinal herbs are an important source for the have enormous physiological activities in humans and therapeutic remedies of various ailments [12]. These include cancer prevention, antibacterial, immemorial, different parts of medicinal herbs have been antifungal, antioxidative, hormonal action, enzyme used to cure specific ailments in Kenya [13].In the Kisii stimulation and many more. Phytochemicals are region, the leave decoction of *Carissa spinarum, Urtica* responsible for medicinal activity of plants and they have *dioica, Warburgia ugandensis, Senna didymobotrya,* protected human from various diseases . *Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* Phytochemicals are basically divided into two groups that and *Toddalia asiatica*, are locally used for the treatment are primary and secondary metabolites based on the of diabetes, malaria and pneumonia [14] . Phytochemicals function in plant metabolism.

The major constituents of are non-nutritive plant chemicals that havprotective or phytochemical are consist of carbohydrates, amino acids disease preventive properties [15]. The plants produce proteins and chlorophylls while secondary metabolites these chemicals to protect themselves but recent research consist of alkaloids, saponins, steroids, flavonoids, demonstrates that they can protect humans and animals tannins and among others [16].The phytochemical constituents are playing a significant role in the identification of crude drugs.

There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects [17] *Amaranthus viridis* belongs to Amaranthaceae family. *Amaranthus*, communally known as Green amaranth or locally as “Karund”, is a multinational genus of herbs. *Amaranthus* or Amaranth is defined as “never-fading flower” in Greek. Several species of *Amaranthus* are often considered as weeds, people around the world worth amaranths as leaf vegetables, cereals and ornamentals [18]. Starchy foods are the main affix of developing coun- tries as they provide both energy and proteins. These accounts in part for protein deficiency which overcomes among the general population are as acknowledged by Food and Agricultural Organization [19].

The pharmacological properties of amaranth products are considered of vital importance [20]. For reducing tissue swelling the leaves are well thought-out to be con- structive, and they have a cleansing effect

too. The plant has also been used curatively for diarrhea, dysentery, excessive menstrual flow, ulcers and intestinal hemor-rhaging. For the treatment of intestinal bleeding, exces- sive menstruation, diarrhea and other related problems, a tea made from its leaves is used [21]. Hence , it is of interst to investigate the aqueous , butanol ,chloroform, acetone ,and aqueous +benzene extract of *Amarathus viridis*

MATERIAL AND METHODS

Collection of samples

The medicinal plant used for the experiment were leaves of Amarathus viridis. The plant parts were identified by the botanist, chennai.

Preparation of extracts

250 grams of dried powder of Amarathus viridis leaves was packed in five separate round bottom flask for sample extraction using five solvents namely aqueous, butanol, acetone, chloroform and ,aqueous and benzene (1:1). The extraction was conducted with 750 ml of each solvent for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phytochemical analysis

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars, tri-terpenoids ,proteins amino acids based on the protocols available in the literature [22-24]

Test for alkaloids

The extract of the crude dry powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrocholoric acid. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The creamish precipitate, the orange precipitate and brown precipitate, indicated the presence of respective alkaloids [25]

Test for saponins

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration [26].

Test for steroids

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoides and orange colour for flavons [27].

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids.

Test for Proteins

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for Amino Acids

To 2ml of sample was added to 2ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Tri-Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H2SO4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

Test for Reducing Sugars

To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of conc. H2SO4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrate

RESULTS AND DISCUSSIONS

Table 1: Shows the phytochemical constituents of aqueous and butyl alcohol extract of *Amarathus viridis*

S.No	Phyto-constituents	Aqueous Extract	Butyl Alcohol Extract
1.	Flavanaoid	+ +	- -
2.	Alkaloids	- -	- -
3.	Saponins	+ +	- -
4.	Tanins	+ +	+ +
5.	Amino acid	- -	- -
6.	Protein	- -	- -
7.	Terpenoids	- -	- -
8.	Reducing sugar	- -	- -
9.	Cardiac glycosides	- -	+ +
10.	Anthroquinones	- -	- -
11.	Steroids	- -	- -

Positive ++ , Negative --

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Table 2: Shows the phytochemical constituents of Acetone and Chloroform extract of *Amarathus viridis*

S.No	Phyto-constituents	Acetone Extract	Chloroform Extract
1.	Flavanaoid	- -	- -
2.	Alkaloids	- -	++
3.	Saponins	+ +	- -
4.	Tanins	- -	+ +
5.	Amino acid	- -	- -
6.	Protein	- -	- -
7.	Terpenoids	- -	- -
8.	Reducing sugar	- -	- -
9.	Cardiac glycosides	++	+ +
10.	Anthroquinones	- -	- -
11.	Steroids	- -	- -

Positive ++ , Negative --

Table 3: Shows the phytochemical constituents of aqueous and benzene extract (1:1) of *Amarathus viridis*

S.No	Phyto-Constituents	Aqueous And Benzene Extract (1:1)
1.	Flavanaoid	+ +
2.	Alkaloids	+ +
3.	Saponins	- -
4.	Tannins	- -
5.	Amino acid	- -
6.	Protein	- -
7.	Terpenoids	- -
8.	Reducing sugar	- -
9.	Cardiac glycosides	- -
10.	Anthroquinones	- -
11.	Steroids	- -

Positive ++ , Negative --

Plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. Hence during the present study. Phytochemical screening of Table 1 shows the phytochemical constituents of aqueous and butanolic extracts of Amarathus viridis. Phytochemical screening of the crude extract revealed the presence of Tanins in both above mentioned extracts whereas the flavonoids and saponins are present in aqueous extract and remaining all negative In the case of cardiac glycosides, cardiac glycosides are present only in butanolic extract and remaining aqueous extract showed negative result whereas alkaloid , tre-terpenoids , reducing sugar , amino acids , anthraquinones , steroids and proteins showed negative result in both aqueous and butanolic extract.

Table 2 shows the phytochemical constituents of acetonic and chloroform extracts of Amarathus viridis. phytochemical screening of acetonic extract showed the presence of saponins and cardiac glycosides whereas absent in flavonoids , alkaloids, tri-terpenoids , tanins , reducing sugar , amino acids , steroids and proteins Whereas the chloroform extract shows the presence of alkaloids, tanins and cardiac glycosides , remaining all viz. flavonoids , tri-terpenoids , reducing sugar , amino acids , steroids and proteins were absent . Table 3 shows the phytochemical constituents of aqueous and benzene extract together in 1:1 ratio of Amarathus viridis . phytochemical analysis of the aqueous and benzene extract (1:1) of Amarathus viridis shows the presence of flavonoids and alkaloids while absent in all remaining viz. tri-terpenoids , tanins and cardiac glycosides , reducing sugar , saponins , anthraquinones, amino acids , steroids and proteins .

The whole of the A. viridis plant is used for the treatment of pain and fever in traditional medicine.However, there is insufficient scientific proof regarding the analgesic and antipyretic activity of *A. viridis,* so our aim is to provide scientific validation fortraditional uses.

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes [28,29,30]. In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe [31]. In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care [32]. There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs.

Since ancient times , peoples have been exploring the nature particularly plants in search of new drugs . This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases [33] Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [34]. In India, almost 95% of the prescriptions were plant based in the traditional systemsof Unani, Ayurveda, Homeopathy and Siddha [35]. The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products were of plant origin and their sales exceeded US $65 billion in 2003 [36].

Amaranthus viridis L (Amaranthaceae) is commonly called 'Chilaka Thota-Kura' in Telugu. *A.viridis* has been traditionally used in India and Nepal to lessen labor pains and as an antipyretic [37]. The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes . Other traditional uses are as an anti-inflammatory agent of the urinary tract, in venereal diseases, as a vermifuge, diuretic, antirheumatic, antiulcer, analgesic, antiemetic; laxative, for improving appetite, as an antileprotic, for the treatment of respiratory problems, eye treatment and for asthma [38]A novel antiproliferative and antifungal lactin and a ribosome inactivating protein, β-carotene, were isolated from A. viridis [39]and it also possesses antiviral activity [40].

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