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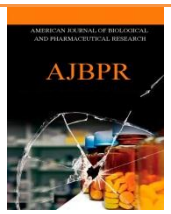

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENTS OF MICROCOCCA MERCURIALIS. (L.) BENTH.

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ABSTRACT

Plants are the chemical factories consist of lots of phytochemicals. Phytochemical are primary and secondary metabolites have some medicinal values and plays an important role in internal mechanisms of plants. The present study was aimed at qualitative and quantitative analysis of phytoconstituents of *Micrococca mercurialis* (L.) Benth. leaf, stem, root and fruit. The shade dried parts of the plant powder were subjected to successive Soxhlet extraction using petroleum ether, benzene, chloroform, methanol and water. These solvent extracts were subjected to the preliminary phytochemical screening to detect the different chemical principles present in the plant. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, quinines, glycosides, steroids, phenols in varying concentrations. The present study provides evidence that solvent extract of *Micrococca mercurialis* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailment cannot be overemphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Plant based drugs have been used worldwide in traditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world [1].

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The use of plant whether herbs, shrubs or tree, in parts or whole in the treatment and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity [2]. During the last century, the practice of herbalism became main stream throughout the world. In spite of the great advances achieved in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medicinal systems. World Health Organization [3]. describes a medicinal plant as any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

Medicinal plants are of great importance to the health of individuals and communities.

Phytochemical study of medicinal plants is base for the discovery of new drugs. Phytochemical studies furnish the basic information regarding the chemical compound present inside the plant materials. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [4]. The most important of these bioactive constituents of plants include alkaloids, tannins, carbohydrates, terpenoids, steroids and flavanoids [5]. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources [6]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [7]. This therefore underscores the need to try as much solvents as possible in screening plant parts for phytochemicals. Primary metabolites are responsible for growth and development of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs. With this background the present study framed to investigate the qualitative and quantitative analysis of phytoconstituents of *Micrococca mercurialis.* (L.) Benth.

MATERIALS AND METHODS

The experimental materials selected for the present study is *Micrococca mercurialis* L. Benth. belongs to the family Euphorbiaceae. The plant material was collected from the campus of St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli District of Tamil Nadu during the month of December 2013. The plant material was identified with the help of SXC herbarium. The shade dried powder was used for determination of characterization and phytochemical analysis.

Phytochemical analysis

Plant material Collection and Solvent Extraction

Mature and healthy plants were collected and washed thoroughly. Every part of the plant (stem, leaves, roots, and fruits) was cut into pieces and was shade dried at room temperature (25-30°c), for about two weeks. The dried plant was ground to fine powder. About 30gm of plant powder was taken in a digestion flask fitted to the Soxhlet apparatus and extracts were obtained successively with petroleum ether, benzene, chloroform methanol and water. These extracts were concentrated and kept in brown bottles were used for the preliminary phytochemical screening [8].

Phytochemical screening

The plant extracts were tested for the presence of bioactive compounds such as Terpenoids, alkaloids, glycosides, steroids, phenols, tannins, flavonoids and Saponins by standard methods [9].

Quantitative estimation of phytochemicals

The following phytochemicals total Carbohydrates, total lipids, pigments [10] total Protein [11], Carotenoids [12] and total phenol [13] were quantitatively estimated.

RESULTS AND DISCUSSION

Phytochemical screening

The plant material was subjected to preliminary phytochemical screening involving successive solvent extraction by different solvents in order of increasing polarity to obtain diverse polar and non polar phytoconstituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents [14]. Knowledge of phytochemical constituents of plant parts is required to understand the basis for any therapeutic effect. Generally the different solvent extracts of *M. mercurialis* leaves, stem, root and fruit indicated the presence or absence of different phytochemicals (Table 1).

Carbohydrates and proteins are important primary metabolites present in plants and it is very essential for the growth and development of the plants. Carbohydrates are present in petroleum ether extract of root, benzene extracts of leaf, stem and fruit, chloroform extract of stem and fruit and methanolic extract of leaf and root. Protein/amino acids are present in petroleum ether extract of leaf and fruit, benzene extract of stem, root and fruit, chloroform extract of leaf, root and fruit, methanolic extract of stem and water extract of leaf and root. Anthocyanin is present in petroleum ether extract of leaf, root and fruit, all the parts of benzene extract, chloroform extract of leaf and stem, methanol extract of leaf, root and fruit and leaf and fruit of water extract. The alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structure types, biosynthetic pathways, and pharmacological activities. The presences of alkaloids contained in plants are used in medicine as aesthetic agents. In the present study alkaloids are present in all the parts of petroleum ether extract and leaf, stem, fruit of benzene extract. In chloroform and water extract, leaf, root and fruit indicated its presence. In methanol extract alkaloids absent only in leaf. Steroid/Phytosteroid is present in petroleum ether extract of root and fruit and benzene extract of stem, root and fruit. The presence of steroids observed in all the parts of chloroform extract. Methanol extracts represent the presence of steroids in all the parts except leaf. The result of water extract symbolize that the steroid is absent only in fruit. Steroids increase the nitrogen level in the body,

thereby producing proteins that help in the production of muscles. It also plays a role in antibacterial activity and regulates the sex hormones. Tannins are present in petroleum ether and methanol extracts of leaf, stem and fruit. In root extract tannin present in benzene solvent only. In water extract it is present in leaf and stem. They are well known antimicrobial agents that could inhibit the growth of microorganisms and can be used against diarrhea [15]. Presence of tannin in this plant could be useful in the treatment associated with heart, anti-inflammatory action, anticoagulant, diarrhea and dysentery [16]. Saponins are present in petroleum ether and methanol extracts of leaf and petroleum ether, benzene, chloroform, water extracts of stem, in root, saponins are present in all the solvents of root and benzene, chloroform and water extracts of fruit. Traditionally saponins have been extensively used as detergents, as pesticides and molluscides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [17]. Saponins have the property of precipitating and coagulating red blood cells [18, 19]. Flavonoids are present in chloroform, methanol and water extract of leaf and all the solvent extracts of stem. In root, flavonoids are present in all the solvent extracts except water. Petroleum ether, benzene, methanol and water extracts indicated the presence of flavanoids in fruit. Flavonoids are the potent water soluble antioxidants and free radicals scavengers, which prevent oxidative cell damage therefore, have strong anticancer activity [19-20]. Moreover, it greatly reduces mortality rates observed in people consuming high levels of plant based foods [21]. Quinones are present in benzene and water extracts of leaf and chloroform, methanol, water extracts of stem. It is also present in all the solvent extracts of root. In petroleum ether and chloroform extracts, fruits only indicated the presence of quinones. Glycosides are present in benzene and water extracts of leaf and chloroform, methanol, water extracts of stem. Petroleum ether, water extracts of root and chloroform, water extracts of fruit indicated the presence of glycosides. Cardiac glycosides are present in benzene, methanol and water extracts of leaf and it is present in all the solvent extracts of stem except benzene extract. It is also present in all the solvent extracts of root and benzene, chloroform, water extracts of fruit. Glycosides are known to lower the blood pressure according to many reports [22]. The presence of this type of phytochemical compounds in the screened medical plants has a wide range of applications and could be certainly used for a variety of applications [6]. Terpenoids were present in benzene, methanol extracts of leaf and chloroform, methanol, water extracts of stem. In root, it is present in all the solvent extracts except water. Petroleum ether, benzene, chloroform and methanol solvent extracts indicated the presence of terpenoids in fruit. Terpenoids are useful in treating cancer as it is an effective antioxidant [23]. Phenols are present in petroleum ether, chloroform and water extracts of leaf, benzene and methanol extracts of stem. In root, it is present in chloroform and methanol extracts. Petroleum ether, methanol and water extracts of fruit indicated the presence of phenols. Phenols and phenolic compounds in the plant indicate that this plant may be used as an anti-microbial agent. This agreed with the findings of [24]. Phenols when mixed with the flavonoid compounds in plants are reported to show multiple activities like antioxidant, anticarcinogenic, anti-inflammatory etc [25].

Phlobatanmins are present in chloroform, water extracts of leaf and benzene, water extracts of stem. It is also represented by petroleum ether, chloroform and methanol extracts of root and benzene, water extracts of fruit. Anthro quinones are present in petroleum ether extract of stem and benzene, methanol extracts of leaf and root. In chloroform extract, it is present in stem and root, fruit extracts of water. Coumarins were present in chloroform, methanol, water extracts of leaf and all the solvent extracts of stem. It is present in all the solvent extracts of root except water. Petroleum ether, benzene and methanol extracts of fruit indicate the presence of coumarins. They are simple phenolic compounds, widespread in vascular plants and appear to function in different capacities in various plant defense mechanisms against insect herbivores and fungi. They are a highly active group of molecules with a wide range of anti-microbial activity against both fungi and bacteria [26].

Quantitative estimation of phytochemicals

The amount of primary metabolites present in different parts of the plant *M. merucrialis* quantitatively determined by standard procedures and the results are displayed in table 2. The various plant parts (root, stem, leaves and fruits) of *M. merucrialis* varied in composition of primary metabolites studied [27-28]. Carbohydrates are one such group of carbon compounds, which are essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life. The carbohydrates content of leaf, stem, root and fruit of (*M. mercurialis*) were 7, 3, 70 and 130mg/g/FW respectively (Figure1). The higher amount of plant lipid can be used as essential oils, spice oleoresins and natural food colors. With a strong foundation in research and development, plant lipids have developed products that work with diverse requirements, be it culinary, medicinal or cosmetics [29]. Lipid content of plant parts were 40, 30, 15 and 80% for leaf, stem, root and fruit respectively (Figure 2). Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [30]. Protein content is more in root (24mg/g/FW) and less

in fruit (0.32mg/g/FW). Leaf and root contains 1.12 and 0.16mg/g/FW respectively (Figure 3).

Several studies have described the anti-oxidant properties of different parts of various medicinal plants which are rich in phenolic compounds [31-33]. Natural anti-oxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols etc [23] and used for the treatment of degenerative diseases. Phenols content is more in leaf (17.2mg/g/FW) and less in stem (10.4mg/g/FW). Root and fruit contains 13.4 and 14.4 mg/g/FW respectively (Figure 4). Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis [34]. The Chlorophyll-a content of leaf, stem and fruit of *M. mercurialis* were 0.6452, 0.076, and 0.06875mg/g/FW. The Chlorophyll-b content of leaf, stem and fruit of *M. mercurialis* were 0.6185, 0.0467, and 0.05771 mg/g/FW. The Total Chlorophyll content of leaf, stem and fruit of *M. mercurialis* were 1.463, 0.1403 and 0.14591 mg/g/FW (Figure 5). The Carotenoids content of leaf stem and fruit of *M. mercurialis* were 78.963, 8.2805 and 9.702 mg/g/FW (Figure 6).

Primary metabolites analysis is necessary for knowing the nutritional potential of plants and also the precursors for the synthesis of secondary metabolites [35].

The present study supported by many workers Pragada *et al*., [36] carried out the preliminary phytochemical analysis and quantification of total phenols of the hydro alcoholic (70% ethanol) extract of *Acalypha indica* and Ramamoorthy *et al*., [37] screened the methanol extract of roots of *Gentiana kurroo* Royle (Gentianaceae) an important and endemic medicinal plant of Kashmir Himalaya for the presence of various bioactive plant metabolites and analgesic activity. The phytochemical analysis revealed the presence of tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates. These results are reminiscent of chief bioactive compound of commercially importance and may perhaps result in great interest in plants pharmaceuticals.

Table 1. Preliminary phytochemical analysis of leaf extract of *M. mercurialis*

S. No	Phytochemicals	Pet. Ether				Benzene				Chloroform				Methanol				Water			
		L	S	R	F	L	S	R	F	L	S	R	F	L	S	R	F	L	S	R	F
1.	Carbohydrates	-	-	+	-	+	+	-	+	-	+	-	+	+	-	+	-	-	-	-	-
2.	Protein/Amino acids	+	-	-	+	-	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-
3.	Anthocyanin	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	-	+
4.	Alkaloids	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	-	+	+
5.	Steroid/Phytosteroid	-	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
6.	Tannins	+	+	-	+	-	-	+	-	-	-	-	-	+	+	-	+	+	+	-	-
7.	Saponins	+	+	+	-	-	+	+	+	-	+	+	+	+	-	+	-	-	+	+	+
8.	Flavonoids	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
9.	Quinones	-	-	+	+	+	-	+	-	-	+	+	+	-	+	+	-	+	+	+	-
10.	Glycosides	-	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	+	+	+	+
11.	Cardiac glycosides	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+
12.	Terpenoids	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	-
13.	Phenols	+	-	-	+	-	+	-	-	+	-	+	-	-	+	+	+	-	-	-	+
14.	Phlobatannins	-	-	+	-	-	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+
15.	Anthro quinones	-	+	-	-	+	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+
16.	Coumarins	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-

(L- Leaf, S-Stem, R- Root, F- Fruit + Presence - absence)

Table 2. Quantitative estimation of different pigments and phytochemicals present in different parts of *M. mercurialis*

S. No	Plant material	Carbohydrates (mg/g/FW)	Percentage of lipids	Protein (mg/g/FW)	Phenol (mg/g/FW)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
1.	Leaf	7	40%	1.12	17.2	0.65	0.6185	1.5	78.96
2.	Stem	3	30%	0.16	10.4	0.09	0.0467	0.14	8.28
3.	Root	70	15%	2.4	13.4	-	-	-	-
4.	Seed	130	80%	0.32	14.4	0.07	0.05771	0.15	9.70

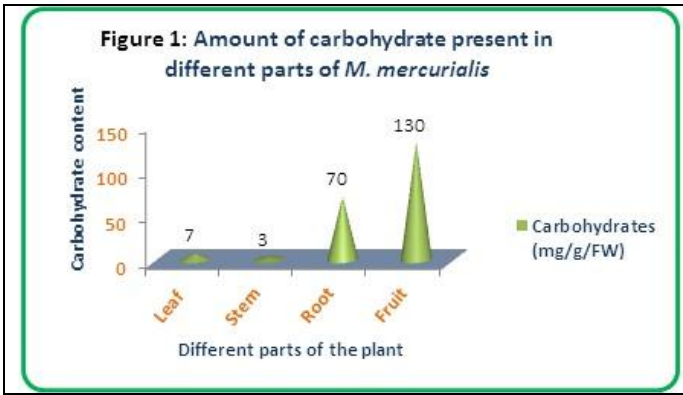

Figure 1: Amount of carbohydrate present in different parts of *M. mercurialis*

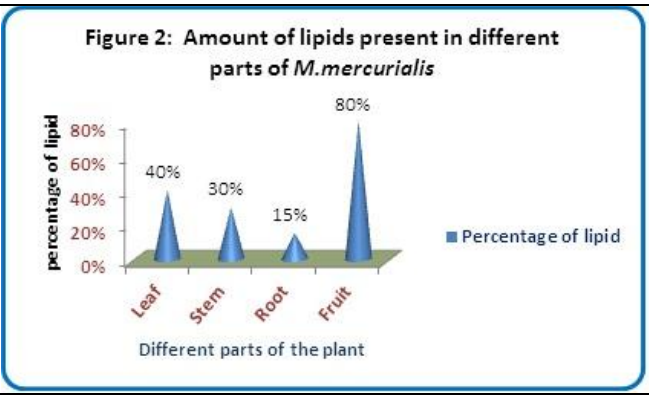

Figure 2: Amount of lipids present in different parts of *M.mercurialis*

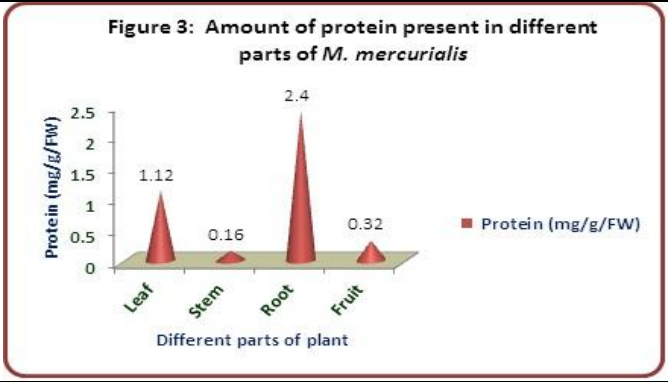

Figure 3: Amount of protein present in different parts of *M. mercurialis*

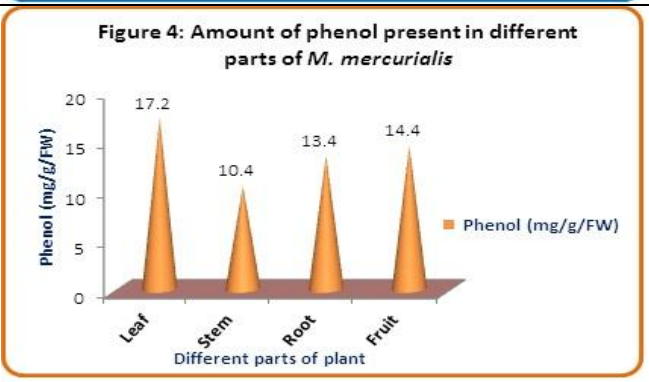

Figure 4: Amount of phenol present in different parts of *M. mercurialis*

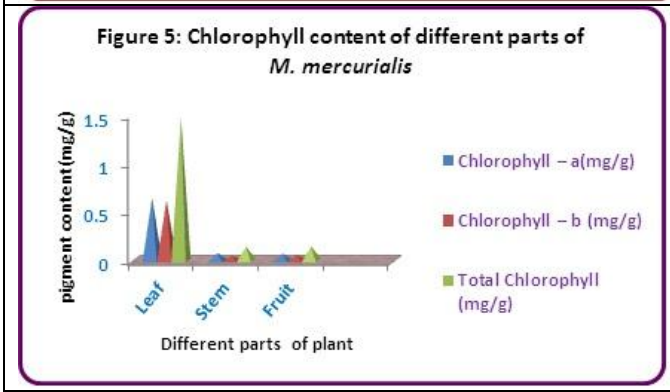

Figure 5: Chlorophyll content of different parts of *M. mercurialis*

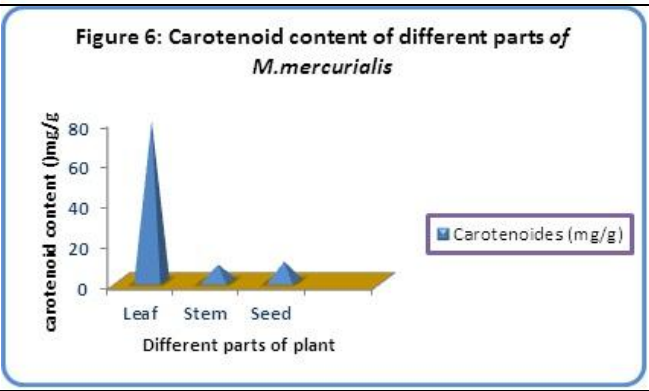

Figure 6: Carotenoid content of different parts *of* *M.mercurialis*

CONCLUSION

The plant based compounds have the effective dosage response and minimal side effects when compared to the synthetic compounds. In the present study, qualitative and quantitative analysis of *M. mercurialis* for the evaluation of phytochemicals were reported. The presence of most general phytochemicals might be responsible for their therapeutic effects. It further reflects a hope for the development of many more novel chemotherapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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