

QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE PHYTOCHEMICAL CONSTITUENTS OF *MOLLUGO* *CERVIANA (L.)*

P .PADMAPRIYA, Dr. S. MANEEMEGALAI

Department of Biochemistry, Bharatihidasan University Constituent College for Women,
Thanjavur, Tamilnadu, India



Date Received:

24-08-2014

Date of Accepted:

24-08-2014

Date Published:

08-Sep-2014

Abstract:

The objective of the present study was to find out the presence of phytochemicals in the methanol extracts of Molluginaceae family such as *Mollugo cerviana* by both qualitative and quantitative screening methods. In qualitative analysis, the phytochemical compounds such as Carbohydrates, Saponins, Tannins, Terpenoids, Flavonoids, Steroids, Phenols, proteins, Alkaloids and absence of Glycosides were screened in extracts by using standard methods. . In quantitative analysis the important secondary metabolites such as alkaloids, flavonoids, Vitamin C and Vitamin E were tested in the extracts. More active compounds will be isolated from the plants and they may be used for medicinal purposes in future.

Keywords: Mollugo cerviana, Methanolic extracts, qualitative and quantitative analysis.

Introduction

Plants have been associated with the human health from time immemorial and they are the important source of medicines since human civilization (Kajaria *et al.*, 2011). Plants still remain one of the major sources of drugs in modern as well as in traditional systems of medicine. Plant secondary metabolites are important sources of many food ingredients and phytochemicals (Dosset *et al.*, 2012). Plants produce several secondary metabolite compounds including alkaloids, cyanogenic glycosides, glucosinolates, flavanoids, saponins, steroids and terpenoids to protect themselves from the continuous attack of naturally occurring pathogens, insect pests and environmental stresses (Kumar *et al.*, 2009; Premkumar *et al.*, 2011). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Nisha Raj *et al.*, 2010). Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals

can protect humans against diseases (Kubmarawa *et al.*, 2008). Many of the plant extracts have proven to possess pharmacological action (Pawar *et al.*, 2001) due to the presence of various phytochemicals. A herbal weed belongs to the family Aizoaceae (Molluginaceae), is also one of the traditionally used medicines, which commonly practiced in many villages of India due to its various beneficial effects. An erect slender annual herb, the herb is considered stomachic, aperients and antiseptic. It is used in stomach ache and to promote local discharge flowers and tender shoots in decoction have a diaphoretic effect roots are used in gout and rheumatism (Nadkarni, 1908; Kirtikar and Basu, 1918). It is considered as a good stomachic, aperient and anti bacterial (Kim *et al.*, 2008). Our present study has been designed with an aim to evaluate the Qualitative and quantitative analysis of the phytochemical constituents of *Mollugo cerviana (L.)* crude methanolic extract.

MATERIALS AND METHODS

Collection of plant materials

The whole plants of *Mollugo cerviana* were collected from Orathanadu Taluk of Thanjavur district in the state of Tamilnadu, India and authenticated by John Britto Rapinat Herbarium, St. Joseph's college, Tiruchirapalli. Then examined carefully old, infected and fungus damaged portion of the plants were removed. Healthy plants were spread out and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder.

Methanol extraction

10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4 °C.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of methanol extracts of *Mollugo cerviana* was conducted following the standard procedures (Brinda et al., 1991).

Quantitative phytochemical analysis

The phytochemicals which are present in the methanol extracts of *Mollugo cerviana* were determined and quantified by standard procedures.

Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415nm. 100µl of the plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates (Kumaran and Karunakaran, 2006).

Determination of total alkaloids

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

Determination of vitamin C:

5 gram of the sample was weighed in to an extraction tubes and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 mins. This was transferred in to centrifuge tubes and centrifuged at 3000 rpm for about 20 mins. It was transferred in to a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette out in to a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% CuSO₄ solution to get a dark end point (Barakat et al., 1973). The amount of Vitamin C was calculated as mg/100 ml of sample.

Determination of vitamin E (Tocopherol)

One gram (1g) of the original sample was weighed, macerated with 20mls of n-hexane in a test tube for 10 minutes and centrifuged for 10 minutes. The solution was filtered, 3mls of the filtrate was transferred into a dry test tube in duplicates and evaporated to dryness in a boiling water bath. Following this, 2mls of 0.5N alcoholic potassium hydroxide was added and boiled for 30 minutes in a water bath. Then 3mls of n-hexane was added and was shaken vigorously. The n-hexane was transferred into another set of test tubes and evaporated to dryness. A volume, 2mls, of ethanol was added to the residue. Another volume, 1ml of 0.2% ferric chloride in ethanol was added. Then 1ml of 0.5% 1,1-dipyridyl in ethanol was added followed by the addition of 1ml of ethanol to make it up to 5mls. The solution was mixed and absorbance taken at 520nm against the blank (AOAC., 1990)

RESULTS AND DISCUSSION

Screening of Phytochemical Analysis

The *Mollugo Cerviana* subjected to preliminary qualitative chemical analysis (Brinda et al., 1991). The preliminary Phytochemical analysis was conducted for the presence of bioactive compounds. The analysis revealed the presence of carbohydrates, saponins, tannins, terpenoids, flavonoids, steroids, phenols, proteins, alkaloids and absence of Glycosides in the whole plant of *Mollugo cerviana* (Table 1).

Quantitative phytochemical analysis

The amount of phytochemicals which are found in the *Mollugo Cerviana* methanolic extract was quantitatively determined by standard procedures. The extracts of *Mollugo Cerviana* showed different amount of various phytochemicals. The flavonoids content was highest followed by alkaloids, Vitamin C and Vitamin E. *Mollugo Cerviana* methanolic extract contained 12.01 mg of alkaloids, 15.06 mg of flavonoids, 8.33 mg of Vitamin C and 4.20 mg of Vitamin E were found (Table 2; Fig 1).

The Phytochemical constituents such as alkaloids, flavonoids, and Vitamins are secondary metabolites of

Table 1: Screening of Phytochemical Constituents of *Mollugo Cerviana*

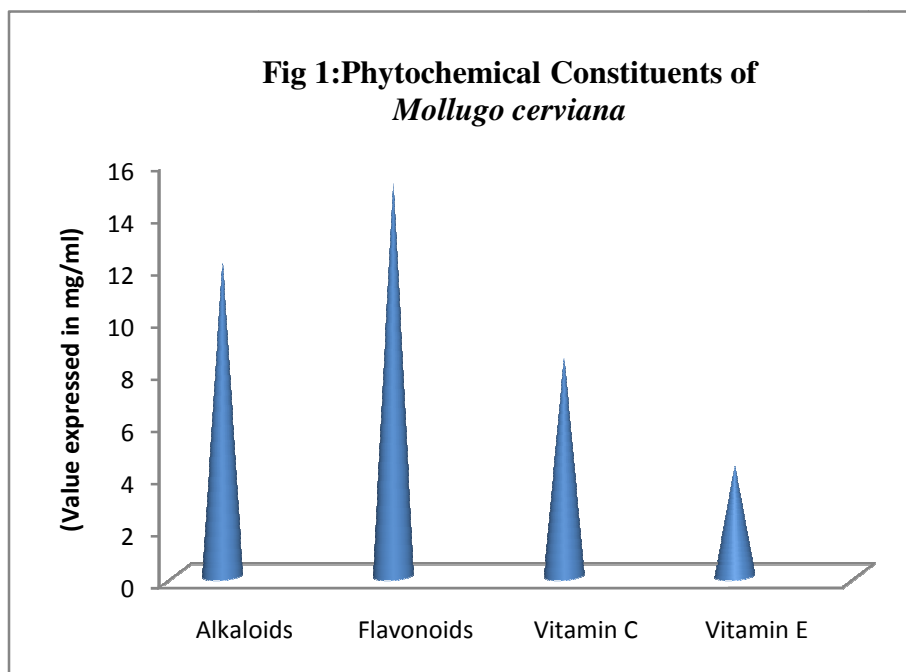
S.No	Phytochemicals	Methanolic Extract
1.	Carbohydrates	+
2.	Saponins	+
3.	Tannins	+
4.	Terpenoids	+
5.	Glycosides	-
6.	Flavonoids	+
7.	Steroids	+
8.	Phenols	+
9.	Proteins	+
10.	Alkaloids	+

+ = Present - = Absent

Table 2:Quantitative Analysis of the Phytochemical Constituents of *Mollugo Cerviana*

Phytochemical constituents	Methanolic extract*
Alkaloids	12.01
Flavonoids	15.06
Vitamin C	8.33
Vitamin E	4.20

Values were expressed in mg/100ml



plants that serve a defence mechanism against many microorganisms, insects and other herbivores (Bonjar et al., 2004).

Flavonoids are known to act as antioxidant by removing the highly unstable molecules called free radicals which damaged the body cells, thereby contributing to a variety of diseases such as cancer, inflammation, heart diseases and aging (Krishnaiah et al., 2009). Flavonoids are responsible for the antipyretic (fever-reducing), analgesic (pain-relieving) and spasmolytic (spasm-inhibiting) properties of these plants (Olaleye, 2007).

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Igbinosa et al., 2009).

Vitamin C, also known as ascorbic acid, is a water-soluble antioxidant, which prevents oxidative damage to the cell membrane induced by aqueous radicals (Sudhakar et al., 2007). Vitamin C is a powerful antioxidant, and acts as a scavenger of ROS to prevent, or at least alleviate, the deleterious effects caused by ROS (Foyer and Noctor, 2005). Acting as a chain breaking antioxidant, it impairs with the formation of free radicals in the process of formation of intercellular substances through the body, including collagen, bone matrix and tooth dentine (Veeru et al., 2009).

Vitamin E is one of the major chain breaking lipophilic antioxidant, within the cell membrane, where it protects membrane fatty acids from peroxidation. Vitamin E has been effective in blocking peroxy mediated chain reaction and in combination with ascorbate, it scavenges $SO^{\bullet}$ in the lipid membrane (Manikandan and Devi, 2005).

The presence of the above discussed medicinally important constituents in *Mollugo cerviana* were responsible for its wide spectrum of pharmacological activity which includes antiseptic, anti-inflammatory, anti-rheumatic and anti-oxidant activities and made it as a handy herbal remedy in treating wounds, inflammation, fever, stomach ache and joint pains.

CONCLUSIONS

In the present study showed the qualitative and quantitative analysis of the phytochemical constituents in the methanolic extract of the whole plant of *Mollugo cerviana* clearly reveals the presence of various biologically active compounds useful in treating various ailments in humans. This study also leads to the further research in the way of isolation and identification of the active compound from the plants using chromatographic and spectroscopic techniques.

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