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***J. Nat. Prod. Plant Resour.*, 2012, 2 (4):512-516**
*(http://scholarsresearchlibrary.com/archive.html)*

**ISSN : 2231 – 3184**
**CODEN (USA): JNPPB7**

# Phytochemical Screening and identification of some compounds from Mallow

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**ABSTRACT**

*The triphytochemical screening of three extracts (etheric, ethanolic and aqueous) of Malva sylvestris L. revealed that the seed contain alkaloids, sterols and steroïds, reducing sugars, tannins, emodols, starch, coumarins and the stem contain flavonoids, tannins, starch, saponins, alkaloids, emodols, sterols and steroids, reducing compounds, coumarins and anthocyanosids which give the medicines several healing properties. The separation of the bioactive compounds from the two parts of the plant extracts was carried out using thin layer chromatography (TLC). However; the aqueous acetone extract of the seed and the stem identified the phenolic compounds such as phenolic acids.*

**Key words:** *Malva sylvestris* L., plant extracts, triphytochemical screening, TLC, phenolic acids.

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## INTRODUCTION

Secondary metabolites are the classes of compounds which are known to show curative activity against several ailments in man, and therefore could explain the use traditional of medicinal plant for the treatment of some illnesses.
There are a chemical compounds (phenolic compounds, alkaloids, terpenoids, steroids, quinones, saponins, etc) with complex structures and with more restricted distribution than primary metabolites. They are not indispensable for the plant that contains them; at least their metabolic functions have not been discovered yet.

According to [1], phenolic compounds is one of the most numerous groups of substances in plant kingdom ranging from simple molecules, such as phenolic acids, to complex compounds, such as tannins. Large groups of phenolic compounds comprises: simple phenols (catechol, resorcinol, etc..), phenolic acids, stilbene (resveratrol, etc..), flavonoids (quercetin, cyanidin, etc..), biflavonoids (ormocarpine, etc..), proanthocyanidins (epicatechin) [2,3], tannins, coumarins and anthraquinones [4,5].

Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. The most common hydroxycinnamic acid derivatives are *p*-coumaric, caffeic and ferulic acids which frequently occur in food as simple esters with quinic acid or glucose [6]. Flavonoids constitute a group of natural compounds that occur in fruits, vegetables, wine, tea, chocolate and other cocoa products. Daily dietary intake of flavonoids and similar polyphenols exceeds that of antioxidative vitamins and provitamins [7].

*Malva sylvestris* L. plant selected for this study is species of mallow belong to family of Malvaceae known as common mallow. It is an annual or perennial herb, growing to a height of four feet [8]. It has been used in folk medicine of Brazil and other countries for the treatment of colitis and stomatitis, in cases of chronic bronchitis, against furuncle and abscess, contusions and haemorrhoids as well as other dolorous and inflammatory processes [9]. In the digestive tract the fruit mucilage can be used to heal and soothe inflammations such as gastritis, peptic ulcers, enteritis, and colitis [8].

The aim of this work is to carry out a phytochemical screening of some extracts of *Malva sylvestris* L. seeds and stems in order to know the composition of secondary metabolites in relation with the structure of their phenolic compounds and better understand the pharmacodynamic properties of its extracts.

## MATERIALS AND METHODS

**Phytochemical screening:**
The triphytochemical tests of seed and stem were analysed after extraction by three solvents (etheric, ethanolic and aqueous). We characterized the different chemicals groups with reference to the technical described in the work of [10, 11, 12, 13].

**1-Test for the phenolic compounds:**
**Flavonoids:**
The ethanol extract (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

**Tannins:**
Two methods were used to test for tannins. First, about 1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration.
Second, 2 ml of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric chloride solution was added . A dark green or blue green coloration indicates the presence of tannins.

**2-Test for saponins:**
To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

**3-Test for alkaloids:**
Three methods were used to test for alkaloids.
-First, evaporate 10 ml of concentred etheric solution, the dry residue was added to 1.5 ml HCl (2%) acid solution. After that, 1 to 2 drops of Mayer's reagent and Wagner was added, and the yellow- white precipitate indicates the presence of the alkaloidal base.
-Second, evaporate 20 ml of ethanol extract, the dry residue dissolved in 5 ml of HCl (2N) and filtered. A few drops of Mayer's reagent and Wagner was added, the presence of precipitate indicates the alkaloids.
-Three, to 15 ml of the aqueous extract was added 2 ml of $NH_4OH$ à 10% (ph=7). The alkaloid was extracted 3 times with 10 ml chloroform. The chloroform layer was washed 3 times with 2 ml of HCL (10%). This was divided into two portions. Mayer’s reagent was added to one portion and Wagner’s reagent to the other. The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids sels.

**4-Test for emodols:**
Evaporate 3 ml of etheric extract. Dissolve the dry residue in 1 ml of concentrated $NH_4OH$ and treating the solution with the reagent Bornträger. A test is revealed by the appearance of a
bright color ranging from orange red to purple.

**5-Test for anthracenosids:**
Eight ml (8 ml) of the ethanolic solution treated with the reagent Bornträger, a positive test is revealed the appearance of a bright color change from orange red to purple.

**6-Test for anthocyanosids:**
The presence of anthocyanosids is revealed by a color change as a function of pH due to titration of the acidic aqueous solution with a solution of NaOH. If the solution turns a red color, the pH is less than 3, if against a blue color; the pH is between 4 and 6.

**7-Test for coumarins:**
Evaporate 5 ml of ethanolic solution, dissolve the residue in 1-2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% $NH_4OH$. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

**8-Test for sterols and steroids:**
Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of ethanolic extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride; we added 0.5 ml of the filtrate chloroforme. Treated with the reagent of Libermann Burchardt. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

**9-Test for the carbohydrat:**
**Reducing sugars** Two methods were used to test for reducing sugars. First, the ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) in a test tube was added too. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars. Second, added to 2 ml of aqueous solution, 5-8 drops of boiling Fehling's solution. A red-brick precipitate showed the presence of reducing sugars.

**Starch**
The aqueous extract 5ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

**Sample extraction:**
The plant materials were dried at room temperature, afterwards samples were ground. Seed and stem of *M. sylvestris* L. were extracted with aqueous acetone (70%, v/v) essentially as described by [14]. The extracts were then washed with hexane to remove chlorophyll and other low molecular weight compounds. The extracts obtained after evaporation (Rotary evaporator-4000-efficient Laborota) were weighed to determine the yield and re-suspended in methanol and stored in a deep freeze before use.

**Chromatographic material**
Thin layer chromatography (TLC) was performed on a silica gel plate (Silica Gel $GF_{254}$, Merck), two microliters (2µl) of each extract and standards were deposited.

Thus we used a mixture of Chloroform / Ethyl acetate / Formic acid: (5: 5: 1) [15] and Cyclohexane / Ethyl acetate / Acetic acid: (31:14:5) [16] as eluents for separation of extracts.

After development of the chromatogram, the plates are dried at room temperature, and the detection is mainly carried out using UV radiation and 365 nm. The colors of the spots and Rf were recorded. Vanillic acid, *para*-coumaric acid, ferulic acid, syringic acid, resorcinol, hydroquinone, phloroglucinol, gallic acid, pyrocatechol, hydrocinamic acid were from Sarsyntex, (France).

## RESULTS AND DISCUSSION

**Phytochemical screening of plant materials**
The triphytochemical screening of the extracts studied of the seed (Table 1) revealed that alkaloïds are present in large amounts with the reducing sugars, sterols and steroids. Other classes are present in small quantities: tannins, emodoles, starch, coumarins. However, the seed extracts tested negative for the presence of anthracenosids, anthocyanosids, saponins and flavonoïds classes.

In the other hand, triphytochemical screening showed that stem (Table 1) contain higher amount of flavonoids and tannins. Starch, saponins, alkaloids, emodols, sterols and steroids, reducing compounds, coumarins and anthocyanosids are present in small quantities. However, anthracenosids classes are absent in the mallow stem extracts.

**Table1: Phytochemical test of seed and stem extracts of Mallow**

| Family of compounds | Ethanol extract | | Etheric extract | | Aqueous extract | |
|---|---|---|---|---|---|---|
| | Seed | Stem | Seed | Stem | Seed | Stem |
| Alkaloids | ++ | + | - | - | ++ | - |
| Tanins | + | +++ | | | + | ++ |
| Flavonoïds | - | +++ | | | | |
| Emodols | | | + | + | | |
| saponins | | | | | - | + |
| Reducing sugars | ++ | + | | | ++ | + |
| Starch | | | | | + | + |
| Sterols and steroïds | ++ | + | | | | |
| Anthracenosids | - | - | | | | |
| Anthocyanosids | + | + | | | | |
| Coumarins | + | + | | | | |

*(-): Negative test (absence of turbidity, flocculation and precipitation).*
*(+): Weak positive test (if the reagent has a slight opacity).*
*(+ +): Positive test (if the reactive product and not a turbidity flocculation).*
*(+ + +): Test strongly positive (if the reagent produces a precipitate or flocculation heavy).*

According to [17], the phytochemical analysis of *Malva sylvestris* L., showed the presence of sterols. And the presence of terpenes is well noted [18]. By cons, [19] noted that *Malva sylvestris* L. contains a scopoletin.

It was noticed that tannins, saponins, flavonoids and carbohydrats were found in the extracts of malva leaves [20]. Literature references about phytochemical screening of seed or stem parts were not found.

**Identified compounds by the TLC**

The separation of the bioactive compounds from two parts of plant extracts was carried out using thin layer chromatography (TLC). The TLC analysis UV light, allowed the identification of a pattern of phenolic acids (blue fluorescent).

For the both seed and stem, we noted the presence of two phenolic acids: syringic acid and *para*-coumaric acid by the 1st solvent system and no compound is identified by the 2nd solvent system (Table 2).

**Table 2: Identified compounds by the TLC**

| Extract | 1st solvent system | 2nd solvent system |
|---|---|---|
| **Seed** | para-coumaric acid<br>syringic acid | - |
| **Stem** | para-coumaric acid<br>syringic acid | - |

*- : not identified.*

Work by [18], revealed 11 phenolic composed that were isolated from water leaf extract of *Malva silvestris* L.. These compounds are: 4-hydroxybenzoic acid, 4 methoxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 2-hydroxybenzoic acid, 4 - hydroxy-2-methoxybenzoic acid, the alcohol 4 -hydroxybenzyl, and tyrosol, as well as: 4-hydroxydihydrocinnamique acid, the 4-hydroxy - 3-methoxydihydrocinnamique acid, 4 - hydroxycinnamic and ferulic acid. The main phenolic acids identified in *Malva sylvestris* L. leaves are gallic, pyrogalllol, vanillic, synergic, cinnamic and chrisin acid [20].

## CONCLUSION

In this study, the seed and stem of *Malva silvestris* L. have a various chemical groups in their chemical composition. It revealed some differences in the constituents of the two parts of the plant tested. Stem extracts tested positive for all except anthracenosids while seed extracts tested positive for all except anthracenosids, anthocyanosids, saponins and flavonoïds . Syringic acid and *para*-coumaric acid were identified by TLC from both parts of plant. Given the importance therapeutic and medicinal of seed and stem extracts, it would be interesting to purify and identify different molecules and tested it in vitro and in vivo by exploiting on animal models, to specify this therapeutic application.

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