PHYTOCHEMICAL SCREENING AND EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF *ACHILLEA MILLEFOLIUM*

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INTRODUCTION

Overview

- The medicinal use of plants is probably as old as Humankind itself. More than 150,000 plant species have been studied, and many of them contain therapeutic substances.

- These substances can be extracted and used in the preparation of drugs, or the plant itself can be used directly as a medication.

Achillea millefolium L. is an example of plants traditionally used for its medicinal properties. Its popular use includes anti-inflammatory, astringent and other actions such as an analgesic, antispasmodic, antiseptic, wound healer and hemorrhoid medication.

It has already been shown that some metabolites are responsible for these pharmacological actions. Therefore, in the present study, the metabolites content of the plant was analytically characterized

THE AIM OF THE PRESENT STUDY

- to investigate the presence of phytochemicals and
- to determine the total phenolic and flavonoid contents of the selected medicinal plants.

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BACKGROUND INFORMATION ABOUT *ACHILLEA MILLEFOLIUM*

Identity

Achillea millefolium is popularly known by “yarrow”.

Is a widespread plant belonging to the Asteraceae family confined to the Northern hemisphere.

- The name of the genus originates from the ancient use as a wound-healing remedy by the Trojan hero Achilles, whereas millefolium refers to the deeply divided leaves.

- It is perennial, erect, aromatical, and a herbaceous plant, of 30-50cm of height. Its leaves are composed, finely pinned, of 5-8cm of length; the flowers are white or rosaceous

CLASSIFICATION OF THE PLANTS:

Kingdom Plantae

Class Magnoliopsida

Order Asterales

Family Asteraceae – Aster family

Genus Achillea L. – yarrow

Species Achillea millefolium – common yarrow

MEDICINAL USES

- The traditional use of yarrow comprises the treatment of inflammatory and spasmodic gastrointestinal disorder.

- Moreover, it is used as an appetite-enhancing drug due to its bitter taste, for wound healing and against skin inflammations.

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METHODOLOGY

Plant collection -

The fresh leaves were collected in May 2017 and they were washed thoroughly and dried in shade for 5 days.

PREPARATION OF EXTRACTS-

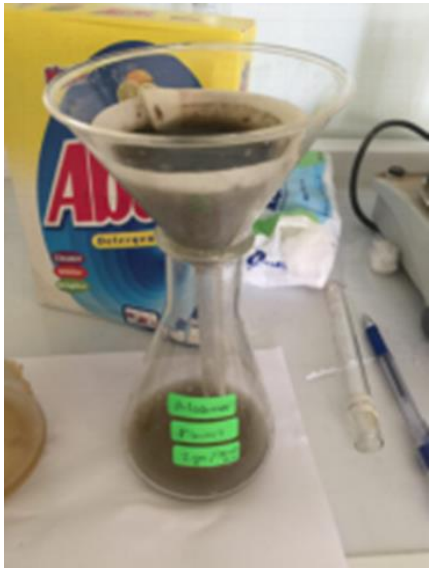
We separate the flowers from the leaves and stems of the plant get two samples. Each one grounded into uniform powder using a manual grinding machine.

The aqueous extract of each sample was prepared:

For the flowers, by soaking 2gm of its dried

powder in 200 ml distilled water into a beaker

and cold-extracted with stirring for 24 hours.



And the leaves and stem by soaking 5 g of dried powdered samples in 200 ml of distilled water for 24 h.

The extracts were filtered by filter paper.

Form each sample we got an extract that was then separated into two equal amounts. One got poured into a petri dish and left for 3 days to get dry. It was then used for further analysis.

The second part was designated the aqueous extract and

preserved for further analysis.

The preparation of the organic extract:

5 g of the powdered leaves and stems were extracted with 200 mL ethanol using Soxhlet extractor, left for 24 hours.

And for the powdered flowers, we extracted 4 gm with 200ml ethanol also by using Soxhlet extractor.

The ethanolic extract s were then collected for further analysis.

PHYTOCHEMICAL SCREENING

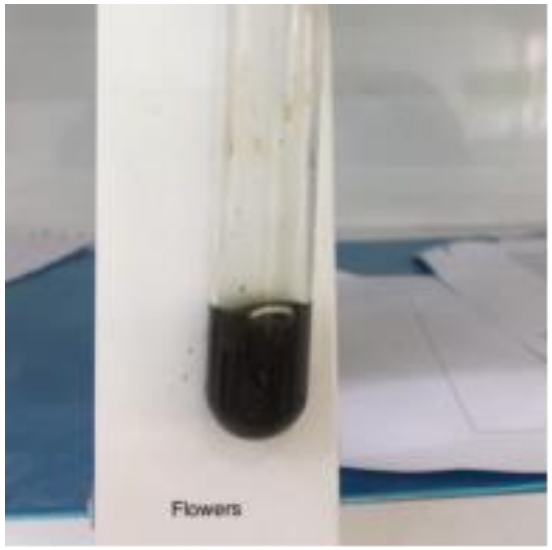
Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents

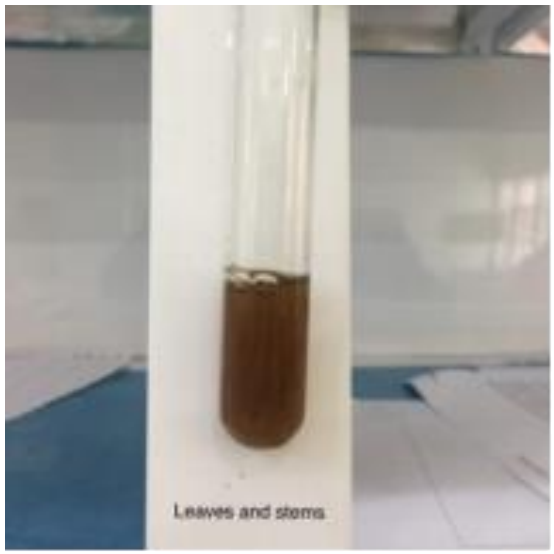
TESTS DONE BY USING THE AQUEOUS EXTRACTS

TEST FOR TANNINS:

2ml of aq. Extract stirred with 2ml D.W. and few drops of $FeCl_3$ solution were added.

a green precipitate indicate the presence of tannins.

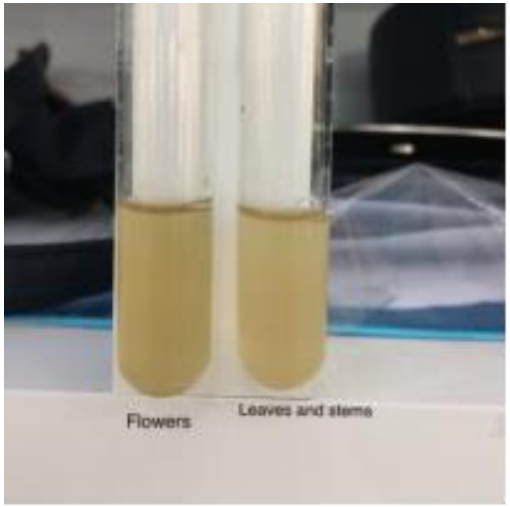




TEST FOR PHLOBATANNINS:

2ml of aq. extract was added to 2 ml of 1% HCL and the mixture was boiled.

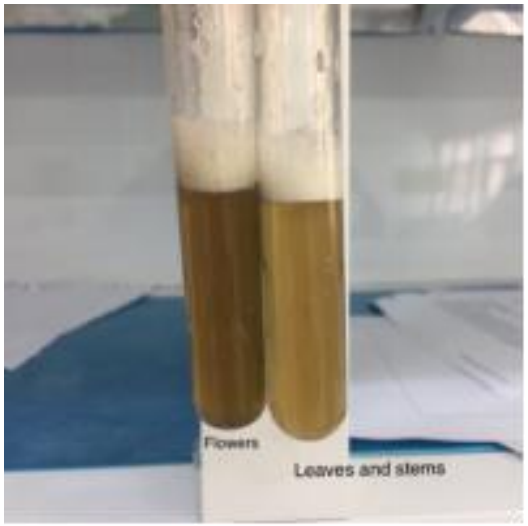
Red precipitate indicate a positive result.



TEST FOR SAPONIN:

The analysis of presence of saponins was done by the formation of foam. 5ml of aquous extract was shaken vigorously with 5ml D.W. in a test tube and wormed.

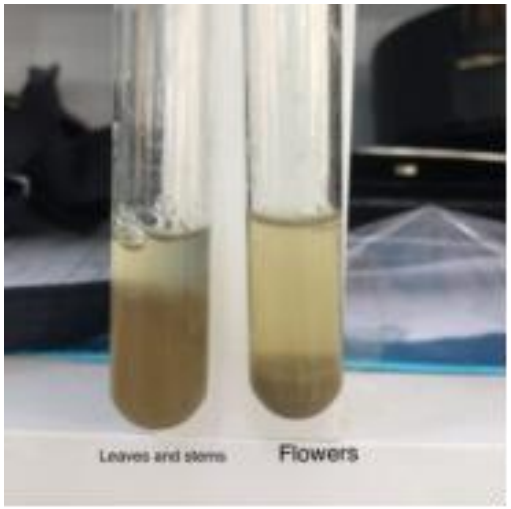
a stable foam indicates the presence of saponins.



TEST FOR FLAVONOIDS:

add 1ml aq. extract to 1 ml of 10% lead acetate solution.

The formation of a yellow precipitate indicate a positive test for flavonoids.



TESTS FOR ANTHRAQUININES:

a) Borntrager's test:

3ml of aq extract was shaken with 3 ml benzene, filtered and

5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken…

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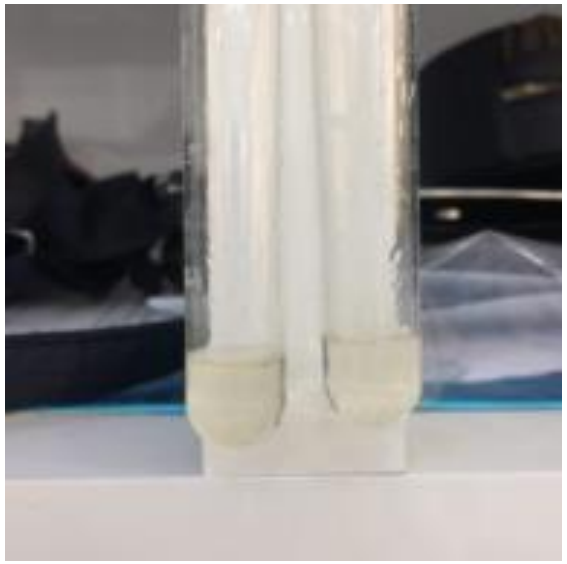


a) 3ml of the aq extract was boiled with 3 ml of aqueous sulphuric acid and filtered while hot. Then 3 ml of benzene was added to the filtrate and shaken.

The benzene layer was separated and 3 ml of 10% NH3 added..

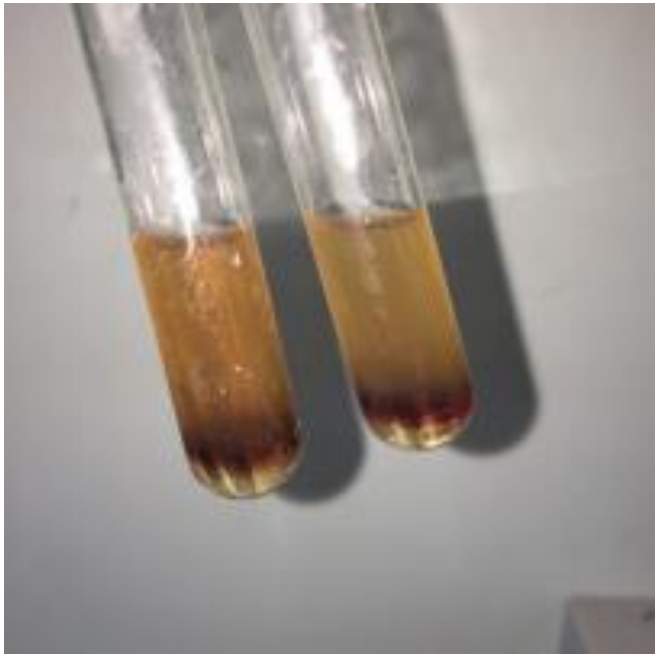
TEST OF CARBOHYDRATES:

Molisch's test:

3ml of the aueous extract was added to 2 ml of molisch's reagent and the resulting mixture shaken properly.

2 ml of conc. H_2SO_4 was then poured carefully down the side of the test tube.

A violent ring at the interphase formed for the two samples indicates the presence of carbohydrate.

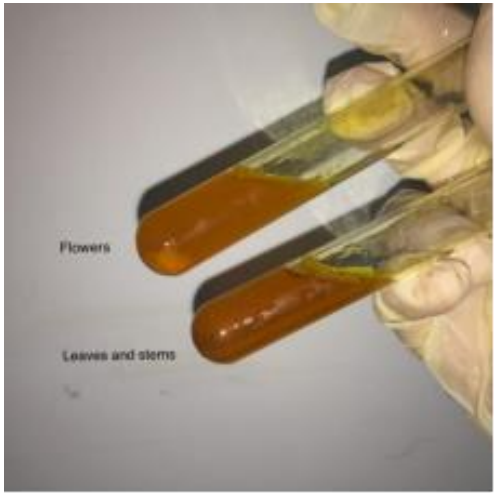


TEST OF ALKALOIDS:

The alkaloids analysis was done by the precipitation reactions with the reagents of Dragendorff,

2 mL of each aqueous extract was treated seperatly with few drops of diluted hydrochloric acid and filtered.

Dragendorff's reagent (solution of potassium bismuth iodide) was added and the formation of an orange brown precipitate indicates the presence of alkaloidsstems



6 . EVALUATION OF THE ANTIBACTERIAL ACTIVITY

To evaluation antibacterial activity of A. millefolium extract, the strains used were Staphylococcus aureus , Staphylococcus epidermidis and Escherichia coli .

The A. millefolium extract was ressuspended in dimethylsulfoxide (DMSO) and diluted (1:1) in brain and heart infusion broth (BHI).

a sample of strains was incubated at 37°C for 24 h in BHI.

After that, 100 µL of bacteria diluted (1:100) was added to the tubes containing extract solution,

All samples were incubated at 37ºC for 24h and the antibacterial activity was observed by the presence of turbidity.

Then a sample of each tube was removed and plated in medium Müeller-Hinton agar which was incubated at 37ºC for 24h to verify bacterial growth.

7. RESULT AND DISCUSION

7.1. PHYTOCHEMICAL SCREENING RESULTS

test	Result for flower Aq. extract	Result for leaves and stems aq. extract
Ferric chloride test	+ve	+ve
Phlobatanin test	-ve	-ve
Saponin test	+ve	+ve
Flavonoid test	+ve	+ve
Borntrager's test	-ve	-ve
Anthraquinone test	-ve	-ve
Dragendroff's test	+ve	-ve
Molisch Test	+ve	+ve

Tables 1: summarize the results of the phytochemical analysis in terms of aqueous extract extract.

7.2 EVALUATION OF THE ANTIBACTERIAL ACTIVITY

The assays were conduced to strains that have importance in Public Heath.

In all tubes of positive control assay of S. aureus, S. epidermidis and E. coli there was growth.

TABLE 2: EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF THE EXTRACT OF A. MILLEFOLIUM

Extract	*A. millefolium*				
Microorganism	100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	12,5 mg/mL
S. aureus	+	+	+	+	+
S. epidermidis	+	+	+	+	+
E. coli	+	+	+	+	+

+ : bacterial growth.

As seen in Table 2,

there was bacterial growth of the strains used in all concentrations of the A. millefolium ethanolic extract. Thus, it was determined that the ethanolic extract does not present antibacterial activity at any concentration assayed. Probably, the absence of antibacterial activity could have as cause, the amount of metabolites as flavonoids and tannins in our samples, because they are compounds described in the literature that presents antimicrobial property

8.CONCLUSION

Based on the results, it was possible demonstrated the absence of anthraquinones in flowers and leaves of A. millefolium.

The results indicated positive results, related to the presence of tannins and saponins, flavonoid and carbohydrates in leaves and flowers aqueous extracts.

the presence of alkaloids had been indicates in A. millefolium flowers aq. extract only.

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- however, of all that reactions had been performed to characterization of this metabolite, only in four, the result was positive. These results can be due to an insufficient sensitivity of the reactions in front of low concentration of these metabolites, or to the place where the plant was cultivated, or even, to the period of collection

- Through the evaluation of the antibacterial activity, it can be verified that the ethanolic extract of the A. millefolium, in the tested conditions, does not present activity against strains of bacteria.