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RESEARCH ARTICLE

Preliminary analysis of Phyto-constituents from the Leaf Extracts of *Ballota nigra Linn*

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ABSTRACT:

Currently, World is witnessing an inimitable growth in the practice of herbal medicines. *Ballota nigra Linn* (Black horehound) of the family Lamiaceae, was found to possess large number of pharmacological values like antioxidants, Neurosedative effect, Hypoglycemic effect, Antimicrobial, insecticidal effect and Anticholinesterase activities. To the best of our knowledge this is first new report on the plant *Ballota nigra* in india. Hence, the objective of the study is to analyse the bioactive constituents from the leaves of *Ballota nigra*. Preliminary phytochemical screening of the leaf extract was carried out with five different solvents of increasing polarity (Hexane, Chloroform, Ethyl Acetate, Methanol, and Aqueous).Qualitative phytochemical analysis of all the extracts was performed by standard methods and it reveals the presence of wide range of Phytoconstituents like Carbohydrates, Tannins, Saponin, Flavonoids, Alkaloids, Quinones, Terpenoids, Glycosides, Triterpenoids, Phenols, Coumarins, Acids, Proteins, Cardiac glycosides and Phytosterols in different deliberations. However, richness of Phytochemicals was observed in methanolic leaf extract. Initial studies will serve as base for further research in evaluating therapeutic potentials of the plant compounds by *in vitro* studies.

KEYWORDS: *Ballota nigra Linn.*, Bioactive constituents, Qualitative, Leaf extract and pharmacological values.

INTRODUCTION:

Plants play momentous part in human health,since ancient times[1]. Medicinal plants have tradtionally served as man's most significant batter against pathogens[2]. WHO,have reported that 80 % of the world populations rely on medicinal plants for their health care system[3,4]. As herbal drug are considering to be more safe and efficacy when compared with synthetic drug. Medicinal plants have resurged the interest for the development of therapeutically effective drugs.

From the 18th century, Scientist started extracting and isolating chemicals from the plants and their effects in terms of the active constituents present in it. Plants are able to synthesize a massive amount of organic molecules/phytochemicals, they are referred to as "secondary metabolites" [5]. Plants are rich source of Phytochemicals which helps in protecting human from various diseases[6]. Secondary metabolites are blended in all parts of plant body – bark, root, stem, leaves, fruits, flowers, and seeds. The therapeutic values of the plant are based on bioactive constituents such as alkaloids, glycosides, steroids and flavonoids, which are potential source of drugs. Acquaintance of Plant constituents is enviable, not only for therapeutic value but also important in disclosing new sources.Successful determination of bioactive constituents from the plants is based upon the solvent used for the extraction methods[7, 8]. *Ballota nigra (Black horehound)* is a perennial herb belonging to the Lamiaceae family, weed in western

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central and northern europe. This is first new report of the plant *Ballota nigra* in india. Aerial parts of the plant are used medicinally to treat different aliments. Leaves of the *Ballota nigra* is used for external wound healing. Internally the plant is used for stomach cramps, whooping cough, nervous disorders, diabetes and gastrointestinal disorders. Significant antioxidant activity was exhibited by leaves[9-14]. Deliberate phytochemical profile of different parts of the plant is desirable to explore the plant for activity and it helps to decide the part(s) for any synergistic evaluation. Hence, the aerial parts of the plant are rich in Phytochemicals. Keeping this view in mind, the present study is carried out in Leaves of *Ballota nigra Linn* for preliminary qualitative analysis using various solvents.

MATERIALS AND METHODS:

Authentication of the plant materials:

The freshleaves of the plant *Ballota nigra Linn* were collected in the month of May in 2014 from Guindy area, Chennai district, Tamilnadu. The plant were identified botanically by Dr. D. Narasimhan Ph.D. in Botany. Medicinal plants, Associate professor, Department of Botany, Madras Christian College (Autonomous), Tambaram. Then, the plants have to be processed for cleaning in order to prevent the deterioration of phytochemicals present in plants.

Preparation of plant material:

About 3 kg of the fresh and tender leaves were collected and washed under running tap water.Then the leaves were shade dried for 15 days.The dried plant material was coarse powdered using an electric blender. Further, the powdered plant material was subjected to extraction using different solvents.

Extraction Procedure:

The successive extraction of 100 g of the powdered sample using nonpolar to polar solvents was done by five different types of solvents such as Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous extract using Soxhlet apparatus. Further, these extracts were collected and concentrated at under reduced pressure using rotary evaporator fig.1. Then the condensed extracts were used for preliminary screening of Phytochemicals.

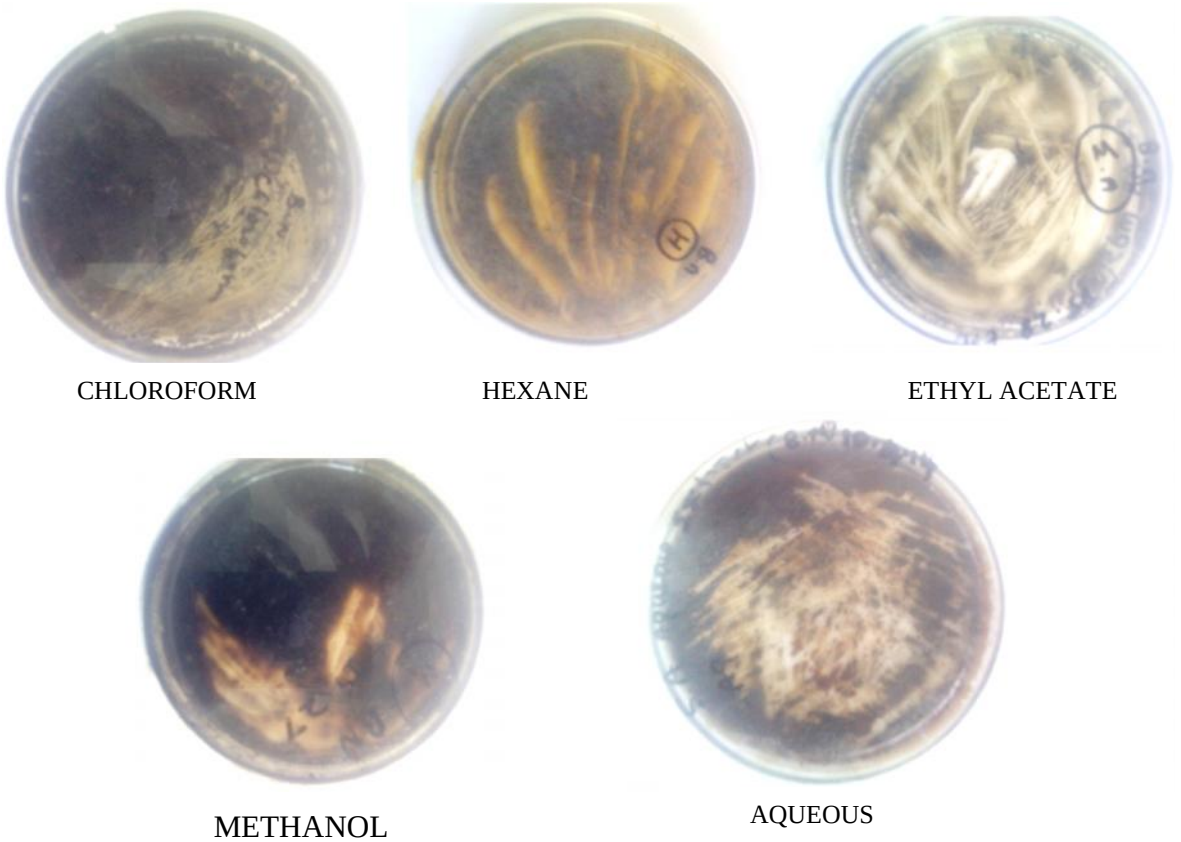

Fig 1: Shows the condensed plate of leaves extract in five different solvent.

Qualitative Phytochemical Analysis:
The solvent extracts was qualitatively examined for the presence of various phytochemical constituents using the standard tests [15-17].

The samples were screened for carbohydrates, alkaloids, flavonoids, phytosterols, phenols, tannins, saponin, glycosides, Quinones, coumarins and proteins etc.

1. Test for alkaloids:
Mayer's Test:
To 2ml of the solvent extracts, 2ml of 1% HCl concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. Formation of green colour or white precipitate indicates the presence of alkaloids

Wagner's Test:
To 2ml of the solvent extracts, 2ml of 1% HCl concentrated hydrochloric acid was added. Then a few drops of Wagner's reagent (solution of iodine in potassium iodide) were added. Formation of reddish brown precipitate which may indicate the presence of alkaloids.

2. Test for carbohydrate:
Molisch's Test:
To 0.5 g of solvent extracts, 1ml of Molish's reagent and concentrated Sulphuric acid was added along the sides of the test tube to form layers. Appeareance of purple or red colour indicates the presence of carbohydrates.

3. Test for tannins:
To 0.5 g of solvent extracts, 1ml of 10%KOH was added. Formation of dirty white precipitate indicated the presence of tannins

Ferric chloride test:
About 1.0 mg of solvent extracts.10 ml of the distilled water was added.from this mixture 2 ml of the all the extracts, 2 drops of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

4. Test for saponins:
Foam test:
To 1.0gm of solvent extracts was taken in ten beakers, 5ml of dilute sulphuric acid was added to five beakers and 5ml of distilled water was added to five beakers. Then they are heated for 2-3min and filtered.5%sodium hydroxide was added to filtrate and heated with Fehling's solution for 3min. The formation of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides 5ml of solvent extract, 5mlof distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

5. Test for flavonoids:
To 2ml of solvent extract, 1ml of 2N Sodium Hydroxide was added. Formation of yellow colour indicates the presence of flavonoids.

6. Test for quinones:
To 1ml of solvent extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones.

7. Test for glycosides:
To 2ml of the solvent extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates the presence of glycosides.

8. Test for terpenoids:
Salkowski's test:
To 0.5ml of solvent extracts, 2ml of chloroform and 1ml of concentrated Sulphuric acid was added carefully. Formation of red brown colour at the interface indicates presence of terpenoids.

9. Test for triterpenoids:
Libermann Buchard Test:
To 1.5 ml of solvent extracts, 1ml of Libermann Buchard reagent (acetic anhydride and concentrated Sulphuric acid) was added. Formation of green colour indicates presence of triterpenoids.

10. Test for phenols:
Ferric chloride Test:
To 1ml of solvent extracts, 2ml of distilled water followed by few drops of 10% Ferric Chloride was added. Formation of blue or green colour indicates the presence of phenols.

11. Test for coumarins:
To 1ml of solvent extracts was treated with 1ml of 10% Sodium Hydroxide. Formation of yellow colour indicates presence of coumarins.

12. Test for proteins and amino acids:
To 2ml of leaves extract, a few drops of 0.2% Ninhydrin was added and heated for 5 minutes. Formation of blue colour indicates the presence of proteins.

13. Test for cardiac glycosides:
To 0.5ml of solvent extract, 2ml of glacial acetic acid and few drops of 5% Ferric Chloride were added. This was under layered with 1ml of concentrated Sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

14. Test for steroids and phytosteroids:
To 1ml of solvent extracts, equal volume of chloroform was added and subjected with few drops of concentrated Sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish ring brown ring indicates the presence of phytosteroids.

RESULT AND DISCUSSION:

Phytochemical investigation on the leaf extracts of *Ballota nigra*, revealed the presence of medicinally important bioactive compounds.

Table1: Result of Qualitative Phytochemical Analysis in the Leaf extracts of *Ballota nigra linn*

S. No	Phytochemical Test	Leaves extract				
		Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract	Aqueous Extract
1	Alkaloids	+++	+++	++	-	-
2	Carbohydrates	+++	+++	-	+++	+++
3	Tannins	-	+	-	+++	++
4	Saponin	-	-	-	+++	++
5	Flavonoids	+++	-	++	+++	++
6	Quinones	-	++	-	+	-
7	Glycosides	-	-	-	+++	-
8	Terpenoids	-	++	+++	+++	++
9	Triterpenoids	-	+++	-	+++	++
10	Phenols	-	+++	-	+++	++
11	Coumarins	+	+	-	-	++
12	Proteins	-	-	-	+	-
13	Cardiac glycosides	-	-	-	+++	-
14	Phytosterols	-	-	++	+++	+

Note : +++ = Strong Positive; ++ = Positive; + = Trace; - = Not detected

Phytochemical compound was evaluated using different solvents such as Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous. Results obtained for qualitative screening of Phytochemicals leaf extracts of *Ballota nigra* in five different solvents are presented in Table 1.

In Hexane extract of the leaf, Flavonoids, Carbohydrates and Alkaloids were found in higher concentration. Coumarins were found in Traces. While Tannins, Saponin, Quinone, Glycosides, Terpenoids, Triterpenoids, Phenols, Protein, Cardiac glycosides and phytosterols were completely absent. In Chloroform extract of the leaf, Secondary metabolites Carbohydrates, Triterpenoids, Phenols and alkaloids exhibits maximum amount where as Quinones and terpenoids showed good but comparatively less than above metabolites. Tannins and coumarins were found in Less content, while the other metabolites were completely absent. In ethyl acetate extract of the leaf, Terpenoids have higher concentrations. Alkaloids and phytosterols showed good while, the flavanoids were present in trace amount. The other metabolites Carbohydrates, alkaloids, Tannins, saponin, quinones, glycosides, Terpenoids, Triterpenoids, Phenols, Protein, Cardiac glycosides and phytosterols were completely absent.In Methanol extract of the leaf, Flavonoids, Carbohydrates, Tannins, saponin, glycosides, Terpenoids, Triterpenoids, Phenols, Cardiac glycosides and phytosterols were found in higher concentrations where as Quinone and protein were found less in content when compared with the above metabolites. Alkaloids and coumarins were completely absent. In aqueous extract of the leaf, Carbohydrates showed maximum amount. Flavanoids, Tannins, Saponin, Terpenoids, Triterpenoids, Phenols, Coumarins and acids showed good but comparatively less than carbohydrates. While phytosterols were present in traces and the other metabolites were completely absent.

Secondary metabolites have been found to acquire a wide range of pharmacological activities, which help to treat various diseases. Phytochemicals such as Flavanoids possess antioxidant activities. phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids, and alkaloids have antiinflammatory effects. saponins possess hypocholesterolemic and antidiabetic properties. Hence, Phytochemical screening of Methanol extract shows huge amount of secondary metabolites such as Flavonoids, Carbohydrates, Tannins, saponin, glycosides, Terpenoids, Triterpenoids, Phenols, Cardiac glycosides and phytosterol.

An in depth study of Phytochemicals will provide through knowledge of pharmacological actions of the plant *Ballota nigra*.

CONCLUSION:

The present work reports the first phytochemical screening activity of *Ballota nigra Linn* in india. Leaves of the plant may be source of Phytochemicals used to treat different types of diseases and disorders.Secondary metabolites present in the plant varies according to parts of the plant. pharmaceutical drug industry mainly rely on the secondary metabolites of the plants for drug development[18-20].Hence, Phytochemical studies had

revealed that the methanolic extract of *Ballota nigra* were found to contain rich source of Flavonoids, Carbohydrates, Tannins, saponin, glycosides, Terpenoids, Triterpenoids, Phenols, Cardiac glycosides and phytosterols. Further, studies are in progress to isolate the active components from the leaves of *Ballota nigra.*

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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