# Studies on, Phytochemicals and antibacterial analysis of *Hemigraphis colorata*, *Marjorana hortensis*, *Artemisia vulgaris*

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## Abstract

Ancient Indian literature incorporates a broad definition of medicinal plants and considers "all" plant entities to be potential sources of medicinal substances. While all plant entities are potentially medicinal, only those plants are considered 'medicinal' whose medicinal use has already been discovered for human or animal application. In the present study under taken the phytochemical analysis and antibacterial activity for three medicinal plants Viz *Hemigraphis colorata, Marjorana hortensis, Artemisia vulgaris* the result shows varies degree of precence of secondary metabolites like alkaloids, carbohydrate glycosides, proteins, saponins, phenols, flavonoids and tannins and the antibacterial activity shows the considerable zone of inhibition activity against gram positive and gram negative bacteria when compare with standard drugs.

## INTRODUCTION:

World is endowed with rich wealth of medicinal plants. Herbs have been the principal form of medicine in ancient India. White herbs lost their importance due to pharmaceutical revolution. They are also becoming popular as people strive to live healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with body's rely defense. Medicinal plants play crucial role in the lives of rural people, in remote parts of developing countries with limited facilities for health care (Purohit and Prajapati, 2003).Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In india thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world. Currently there is a phenomenal increase in screening medicinal plant preparations as a safe alternative to conventional medicines. Genetic analysis of various

characters is relatively difficult in some of the medicinal plants due to its prolonged reproductive cycle and also some of them are dioecious nature which prevents selling. This study was, planned to explore phytochemical constituents, antibactrial analysis studies of three different medicinal plants collected from Ramnagara such as *Hemigraphis colorata*, *Marjorana hortensis*, *Artemisia vulgaris*, selected from ethno botanical survey

## Materials & methods

## Collection of plant materials

The medicinal plants will be identified by ethno botanical survey and from traditional healers of Ramnagara, Karnataka and collected for the experimental purpose. The plants and the parts screened, together with their families and vernacular names. Fresh plant material will be washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottle for further studies. The plants shortlisted for the present investigation are as *Hemigraphis colorata*, *Marjorana hortensis*, *Artemisia vulgaris*,

**Phytochemical analysis:**

Extracts will be tested for the presence of alkaloids, tannins, flavonoids, polyphenols and sapnins etc using standard procedures (Kokate 1994). 1:10 dilution of extracts was prepared and then subjected to phytochemical screening of Glycosides, Alkaloids, Steroids and triterpenoid, Flavonoids, Phenols and Saponins

**Tests for Alkaloids:**

a) **Dragendorffs test:** To 1 ml of test solution, few drops of Dragendorffs reagent was added and observed for orange brown precipitate.

b) **Mayor's test:** To 1-3 ml of test solution, few drops ofMayer's reagent was added and observed for the precipitate.

c) **Hager's test:** To 1-3 ml test solution, Hager's reagent was added and observed for yellow precipitate.

d) **Wagner's test:** To 1-3 ml test solution, few drops of Wagner's reagent was added and observed for reddish brown precipitate.

**4.3.2 Test for Saponins:**

a) **Foam test:** The test solution was shaken and observed for the formation of foam,

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which has to be stable for at least 15 min.

b) **Haemolysis test:** 2 ml of 18% sodium chloride was taken in 2 test tubes. To one test tube distilled water and to the other 2 ml test solution was added. A few drops of blood were added to both the test tubes. They were mixed well and observed for haemolysis under the microscope.

**Test for Glycosides:**

a) **Baljet's test:**

The test solution was treated with sodium picrate and observed for yellow to orange colour.

**b) Legal's test (For cardenoloids):** To the test solution, 1ml pyridine and 1 ml sodium nitroprusside were added and observed for pink to red colour.

**c) Test for deoxysugars (Kellar Killani test)** To 2 ml test solution, glacialaceticacid,one drop of 5% FeCb and concentrated H2S04 were added and observed for reddish brown colour at the junction of the two liquids and a bluish green upper layer.

**d) Test for anthraquinone glycosides:**

**Borntrager's test:**

The powdered drug was mixed with 5 ml of 10% sulphuric acid for 5 mins. It was filtered while hot. The filtrate was cooled and shaken gently with equal volume of benzene. The benzene layer was separated and then treated half of its volume with solution of ammonia (10%). They were allowed to separate. The ammoniacal layer will acquire rose pink colour ifanthraquinones are present.

**Tests for Carbohydrates:**

**Preparation** of test **solution:**

The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of2N HC1 and subjected for the following chemical tests.

a) **Barfoed's test:**

b) Equal volumes of Barfoed's reagent and test solution were takenanheated for 1-2 minutes in a boiling water bath and cooled. It was then observed for a red precipitate.

b) **Fehling's test:** 1 ml Fehling's A and 1ml Fehling's B solutions were mixed and boiled for one minute. To this solution an equal volume of test solution was added and heated in a boiling water bath for 5-10 min and observed for a brick red precipitate.

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**Tannins and phenol compounds:**

To 2-3 ml extract solution, few drops of following reagents were added:

a) **5% FeC13 solution:** and observed for deep blue-black colour.

b) **Lead acetate solution:** Observed for white precipitate.

c) **Bromine water:** Observed for the discoloration of bromine water.

d) **Acetic acid solution:** Observed for red colour.

e) **Dilute iodine solution:** Observed for a transient red colour.

f) One drop of $NH_4OH$ and excess 10% $AgNO_3$ solution were added to the test solution and heated for 20 min in a boiling water bath. White precipitate will be observed and later dark silver mirror deposits on the walls of the test tube.

**Tests for Proteins:**

a) **Ninhydrin test:** 3 ml of test solution and 3 drops 5% Ninhydrin solution were heated in a boiling water bath for 10 min. It was observed for purple or bluish colour.

b) **Million's test:** 3 ml of test solution was mixed with 5 ml of Million's reagent, and observed for white precipitate. This precipitate when warmed turns brick red or it will dissolve giving red colour.

c) **Biuret** test: To 3 ml test solution, 4% NaOH and few drops of 1% CUS04 solution was added and observed for violet or pink colour.

**Tests for Steroids:**

**Preparation of test solution:** The extracts were reflux separately with alcoholic solution of potassium hydroxide till complete saponification occurs. The saponified extract was diluted with water and unsaponifiable matter was diluted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following tests by dissolving the residue in Chloroform;

a) **Salkowiski test:** To 2 ml of extract solution, 2 ml chloroform and 2 ml concentrated H2S04 were added. They were shaken well, and observed whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence.

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b) **Libermann-Burchard test:** 2ml of the extract was mixed with chloroform and 1-2 ml of acetic anhydride and 2 drops of concentrated H2SC>4 were added to the sides of the test tube. First red, then blue and finally green colour can be observed[69].

**Antibacterial Activity:**

Preparation of culture media : Muller Hinton Agar ( HIMedia) is used for the antibacterial susceptibility study The ingredients are weighed and added in 1000ml distilled water and boiled to dissolve it completely. The pH of media was adjusted to 7.4 = 0.2 (at 25 c) and sterilized it by autoclaving at 15 lbs pressure (112C) for 15 min. The solution of the test extracts was prepared at the concentration of 5 mg/ml by dissolving in dimethylsulphoxide (DMSO) in the stopper specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator one hour prior to use and is allowed to warm up to room temperature.

**Screening for antibacterial properties**

Antimicrobial activities of different extracts is evaluated by the agar well diffusion method ( Murray P R et al., 1995) modified by (Olurinola P F, 19)

**Antibacterial Screening of Extracts**

**Microorganisms:** Bacterial strains were obtained from National collection for Industrial Microorganisms( NCIM). The following Gram-positive, Gram-negative bacteria were used in the present study to determine the antibacterial activity of the 3 plants extracts.

| |
|---|
| *C.coagulans* (NCIM-2313) |
| *B. subtils* (NCIM-2063) |
| *S. aureus* (NCIM-2079) |
| **Gram negative bacterial organisms** |
| *E. Coli* (NCIM-2063) |
| *K. pneumonia* (NCIM-2249) |
| *P. aeruginosa* (NCIM-2200) |

The bacterial stock cultures were maintained on Nutrient agar which were stored at 4°C.

**Antibacterial Activity**

**A. Preparation of culture media[70,71]**

**a) Nutrient Agar Medium**

The Nutrient Agar medium was prepared by dissolving 28 g of nutrient agar in 1000 ml of distilled water.

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| | | | |
|---|---|---|---|
| Tannins | + | + | + |

In the present study 3 medicinal plants viz. *Hemigraphis colorata, Marjorana hortensis, Artemisia vulgaris,* were extracted through methanol . The methanol extract of *Hemigraphis colorata* shows the presence of alkaloids, carbohydrate glycosides, proteins, saponins, phenols, flavonoids and tannins were present , steroids and steols is absent. where as in ***Marjorana rtensis*** carbohydrate glycosides saponins, phenols, flavonoids and tannin is present, Protein & Amino Acid steroids and steols is absent. ***Artemisia vulgaris*** shows the precence of alkaloids, carbohydrate glycosides, proteins, saponins, phenols, steroids, tannins flavonoids and steols were found to be absent.

**Antibacterial Activity**

**Table 2. Data Showing the Anti-bacterial activity of methanol extract of *Hemigraphis colorata, Marjorana hortensis, Artemisia vulgari* and standard antibiotic Pencillin against Gram positive Bacteria.**

| Samples | Conc.(µg/ml) | Diameter of zone of Inhibition (mm) | | |
|---|---|---|---|---|
| | | B. coagulans | B. subtils | S. aureus |
| **I.Plant Extract** | 62.5 | -- | -- | -- |
| 1 *Hemigraphis colorata,* | 125 | $9.6\pm0.5^{c}$ | $8.3\pm0.6^{c}$ | $9.4\pm0.4^{c}$ |
| | 250 | $12.6\pm0.5^{b}$ | $10.3\pm1.2^{c}$ | $13.6\pm0.6^{c}$ |
| | 500 | $14.25\pm1.0^{b}$ | $12.3\pm0.5^{a}$ | $19.2\pm1.1^{c}$ |
| | 1000 | $17.3\pm0.6^{a}$ | $16.6\pm0.6^{a}$ | $21.8\pm1.4^{a}$ |
| 2 *Marjorana hortensis,* | 62.5 | -- | -- | -- |
| | 125 | $8.3\pm0.5^{c}$ | $8.3\pm0.5^{c}$ | $9.3\pm1.1^{c}$ |
| | 250 | $12.3\pm0.3^{c}$ | $10.3\pm0.5^{b}$ | $13.2\pm0.4^{b}$ |
| | 500 | $14.4\pm0.8^{b}$ | $12.3\pm0.1^{b}$ | $19.3\pm0.5^{a}$ |
| | 1000 | $16.6\pm1.4^{a}$ | $14.6\pm0.4^{a}$ | $21.6\pm0.8^{a}$ |
| 3*Artemisia vulgari* | 62.5 | -- | -- | -- |
| | 125 | $8.2\pm1.4^{c}$ | $8.2\pm1.1^{c}$ | $9.6\pm0.5^{c}$ |
| | 250 | $9.3\pm0.5^{c}$ | $9.33\pm1.4^{b}$ | $14.3\pm0.1^{b}$ |
| | 500 | $12.6\pm1.0^{b}$ | $11.6\pm0.4^{b}$ | $19.6\pm0.4^{a}$ |
| | 1000 | $16.3\pm1.2^{a}$ | $17.3\pm0.5^{a}$ | $21.3\pm1.0^{a}$ |
| **II. Standard Drug** | | | | |
| a) Pencilin | 100 | 18.3±0.57 | 15.8±0.10 | 20.6±0.57 |

- Values are mean ± SD of three separate experiments.
- --- No Inhibition Zone.

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- Statistical value $^{a}P<0.000$; $^{b}P<0.01$; $^{c}P<0.05$ when compared to the standard.

**Table 3. Data Showing the Anti-bacterial activity of methanol extract of *Hemigraphis colorata, Marjorana hortensis, Artemisia vulgari* and standard antibiotic Pencillin against Gram negative Bacteria.**

| Samples | Conc.(µg/ml) | Diameter of zone of Inhibition (mm) | | |
|---|---|---|---|---|
| | | *E. Coli* | *K. pneumonia* | *P. aeruginosa* |
| **I.Plant Extract** | 62.5 | -- | -- | -- |
| 1 *Hemigraphis colorata,* | 125 | $8.6\pm0.5^{c}$ | $8.3\pm0.6^{c}$ | $9.4\pm0.4^{c}$ |
| | 250 | $11.6\pm0.5^{b}$ | $11.3\pm1.2^{c}$ | $12.6\pm0.6^{c}$ |
| | 500 | $13.25\pm1.0^{b}$ | $11.3\pm0.5^{a}$ | $18.2\pm1.1^{c}$ |
| | 1000 | $15.3\pm0.6^{a}$ | $15.6\pm0.6^{a}$ | $20.8\pm1.4^{a}$ |
| 2 *Marjorana hortensis,* | 62.5 | -- | -- | -- |
| | 125 | $8.3\pm0.5^{c}$ | $8.3\pm0.5^{c}$ | $9.3\pm1.1^{c}$ |
| | 250 | $11.3\pm0.3^{c}$ | $9.3\pm0.5^{b}$ | $12.2\pm0.4^{b}$ |
| | 500 | $13.4\pm0.8^{b}$ | $11.3\pm0.1^{b}$ | $18.3\pm0.5^{a}$ |
| | 1000 | $17.6\pm1.4^{a}$ | $13.6\pm0.4^{a}$ | $20.6\pm0.8^{a}$ |
| 3*Artemisia vulgari* | 62.5 | -- | -- | -- |
| | 125 | $8.1\pm1.4^{c}$ | $8.2\pm1.1^{c}$ | $9.6\pm0.5^{c}$ |
| | 250 | $8.3\pm0.5^{c}$ | $9.33\pm1.4^{b}$ | $13.3\pm0.1^{b}$ |
| | 500 | $11.6\pm1.0^{b}$ | $12.6\pm0.4^{b}$ | $17.6\pm0.4^{a}$ |
| | 1000 | $15.3\pm1.2^{a}$ | $16.3\pm0.5^{a}$ | $25.3\pm1.0^{a}$ |
| **II. Standard Drug** | | 18.3±0.57 | 16.8±0.10 | 20.6±0.56 |
| b) Gentamycine | 100 | | | |

- Values are mean ± SD of three separate experiments.
- --- No Inhibition Zone.
- Statistical value $^{a}P<0.000$; $^{b}P<0.01$; $^{c}P<0.05$ when compared to the standard.

In the present study a variety of Gram positive and Gram negative bacterial stains were selected for the screening of antibacterial activity exhibited by the 3 selected plant extracts to perceive their antibacterial spectrum and to find out the various concentration's which the 3 plant extracts showed activity. **Table's No.2 and 3** show the inhibitory effects of 3 plants

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