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A Comparative study of Phytochemical Screening in Leaf Extracts of *Andrographis Paniculata* collected from Different Geographical Areas

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ABSTRACT

Phytochemicals are secondary metabolites produced by all plants in which some has medicinal uses. Andrographis paniculata is an important medicinal plant belongs to the family Acanthaceae. It is being used in traditional medicine, as a remedy for the cold, fever and detoxification of the body since time immemorial. The present study is taken up to compare the phytochemicals in the leaf extract and to select the elite species of Andrographis paniculata collected from three geographical locations with different edaphic and climatic conditions in Telangana State, India. Fourteen phytochemicals were tested and the extractions were prepared in aqueous, acetone, ethanol, petroleum ether and chloroform. The results from the present study shown that the maximum concentration of alkaloids were observed in plants collected from Osmania University campus area when compared to plants of other two sites, in contrast to this glycosides are completely absent in the plants collected from the Ananthagiri forest area. From the study one can collect the plants from the region where it is showing maximum concentration of phytochemicals and gives a scope for further study to extract pure compound from the plant.

KEY WORDS: *Andrographis paniculata, medicinal plant, Phytochemicals, geographical region, Telangana state.*

INTRODUCTION

Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils [1]. India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Herbal drugs have great importance in modern days for the treatment of various ailments [2]. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives [3]. According to an estimate, 120 or so plant based drugs prescribed for use through the world come from just 95 plant species [4]. The present study was conducted with the objectives to compare the phytochemicals present in ecotypes of *Andrographis paniculata* growing in different geographical areas with varied climatic and edaphic conditions.

Andrographis paniculata of the family Acanthaceae the much branched annual herb. The plant is distributed throughout the tropics. It is found in the plains of India in Assam, Uttar Pradesh, Madhya Pradesh, Telangana, Andhra Pradesh, Tamil Nadu and Kerala states [5]. The plant is an erect annual herb to a height of 30–110 cm in moist, shady places, extremely bitter in taste in all parts of the plant body. The lance-shaped leaves have hairless blades measuring up to 8 centimeters long by 2.5 wide. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles.

MATERIALS AND METHODS

Collection and authentication of plant materials:

The leaves of the plant species were collected wildly from three geographical areas of site-I. Hyderabad (17.411^{0}N78.529^{0}E), site-II. Narsapur (17^{0}44'19"N78^{0}16'58"E) and site-III. Ananthagiri (17.33^{0}N77.90^{0}E). The collected plants were identified using available published literature [6-8] at the Department of Botany, Osmania University, Hyderabad, India

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Preparation of extracts:

To prepare the acetonic, ethanolic, chloroformic and petroleum ether extracts, 150 g of each plant material was collected, dried in the oven at 70^{0}C for 4 hrs and grinded to powder. It was separately macerated with the above solvents and allowed to stand for 72 hrs and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use. For the aqueous extraction, 50 g of the plant powder was weighed into 50 ml Eylen-Mayer flask and to this was added 400 ml of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered with No. 1 Whattman filter paper (W and R Balson Ltd, England). The filtrate was then filtered, sterilized using a membrane filter of pore size 0.45 cm diameter (millipores corp, England). The extracts were concentrated in a hot water bath at 80^{o}C for 5 hrs during which 0.5 g charcoal was added to decolorize it. Sterile decolorized filtered extract was then refrigerated at 5^{0}C until required for use.

Phytochemical screening:

Chemical tests for the screening and identification of phytochemical constituents in the medicinal plants collected from three regions were carried out in leaf extracts using the standard procedures as described by [9-11].

Test for Flavonoids: 0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of Flavonoids.

Test for alkaloids: 0.5 to 0.6 g of various extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Test for Glycosides: Five ml each of various extract were hydrolyzed separately with 5 ml each of conc. HCl and boiled for few

hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

Test for steroids: 0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Phenols: To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

Test for Terpenoids (Salkowski test): 5 ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for Saponins: 0.5 g of various solvent extract was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponins contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Test for Resins: One ml of various solvent extract was treated with few drops of acetic anhydride solution followed by one ml of conc. H_2SO_4. Resins give colouration ranging from orange to yellow.

Test for Tannins 0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Test for Cardiac glycosides (Keller-Killani test) 5 ml of various solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Test for Carboxylic acid: One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.

Test for Coumarins: 0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Test for Quinones One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for Xanthoproteins One ml each of the various extract was treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of Xanthoproteins.

RESULTS AND DISCUSSIONS

From the present study of comparative evaluation of phytochemicals from leaf extracts in various solvents has shown considerable variations in above mentioned 14 phytochemicals. The detailed investigations of phytochemicals in the plants collected from different areas are shown in **Table 1**. The results from the present study are as follows:

Flavonoids: The maximum presence of Flavonoids are observed in the leaf extracts of plants collected from site-II and site-III in aqua and ethanol leaf extracts, whereas in plants collected from site-I they are minimum in presence.

Alkaloids: They are maximum present in plants collected from site-I in all the polar and non-polar solvents, but alkaloids are observed minimum in plants of other two site.

Steroids: Maximum presence is in the plants of site-I in aqua and ethanol leaf extracts, while it is minimum in site-II and III.

Phenols: Phenols are observed maximum in plants collected from all the sites I, II and III in aqua and ethanol leaf extracts.

Terpenoids: This group of phytochemicals are observed maximum in plants of site-II in aqua and chloroform leaf extracts, whereas they minimum in site-I and site-III plants.

Saponins: These are maximum in plants collected from site-II in acetone leaf extracts, but they are absent in other two sites.

Resins: Maximum presence of resins are observed in site-I and III plants in aqua leaf extracts, whereas they are absent in plants of site-II.

Tannins: This group are maximum in plants collected from site-I and II in aqua, acetone and chloroform leaf extracts, while tannins are absent in site-III plants.

Cardiac glycosides: They are observed maximum in plants of site-I in aqua, acetone and petroleum ether leaf extracts, but they are present minimum in other two sites.

Xanthoproteins: Maximum Xanthoproteins are observed in plants collected from site-I and II in aqua, chloroform and ethanol, whereas they absent in site-III plants.

A remarkable observation is that the glycosides, carboxylic acid, coumarins and quinines are completely absent in plants collected from all the three sites.

Table No. 1: showing the comparative phytochemical screening in plants from three sites

S.No.	Name of the Phytochemicals	SITE-I	SITE-II	SITE-III
1	Flavonoids	Min	max	max
2	Alkaloids	Max	min	min
3	Glycosides	Min	min	min
4	Steroids	Max	min	min
5	Phenols	Max	max	max
6	Terpenoids	Min	max	min
7	Saponins	absent	absent	absent
8	Resins	Max	max	max
9	Tannins	Max	min	absent
10	Cardiac glycosides	Max	min	min
11	Carboxylic acid	absent	absent	absent

12	Coumarins	absent	absent	max
13	Quinines	absent	absent	absent
14	Xanthoproteins	Max	max	absent

CONCLUSIONS

Thus, from the present study on comparison of phytochemical screening in plant *Andrographis paniculata* collected from three different geographical areas shown noteworthy and remarkable observations. From the above study it is clear that the plant is producing abundant phytochemicals as secondary metabolites in different climatic and edaphic conditions which can be used to produce a potent drug. The plants collected from site-I are showing maximum presence of phytochemicals while the plants from site-III are showing minimum phytochemicals. Production of some phytochemicals in one site and absence of the same in another site may be due to environmental induced production of certain phytochemicals or may be due to activation or suppression of certain genes producing the phytochemicals in particular environmental conditions and further which gives a scope for further investigations. Hence, out of the plants collected from the above three site, the site-I plants can be selected to extract maximum number of phytochemicals.

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