QUALITATIVE ANALYSIS OF ANDROGRAPHIS PANICULATA NEES

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ABSTRACT

Andrographis paniculata Nees is a plant that has been effectively used in traditional Asian medicines for centuries. It’s perceived “blood purifying” property and also for medicinal properties. The present paper deals to study the phytochemical screening of Andrographis paniculata for various medicinally important compounds. In the present study different parts (leaves, stem, root) were analyzed by using different solvent. From preliminary analysis, it was found that phenols, alkaloids, tannins, flavanoids are present in leaves, stem and root of the plant. Saponin is absent. The methanol and ethanol extracts of the each part showed positive results for most phytochemicals while chloroform extracts of different parts showed less phytochemicals. Presence of tannin, alkaloids, flavonoids, terpenoids, etc. revealed more pharmacological activity.

KEY WORDS: Andrographis paniculata, Phytochemicals, secondary metabolites.

INTRODUCTION

Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations of plants for their biological effects in human beings. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal product to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people (Taylor, et.al.2003). India has been identified as a major resourceful area in the traditional and alternative medicines globally. Medicinal plants constitute an important component of flora and are wildly distributed in India. Andrographis paniculata Nees is one of the wildly distributed medicinal plants in India and using since ancient times in traditional ayurvedic systems of medicines. Andrographis paniculata Nees is a medicinal plant belonging to the family of Acanthaceae. Diterpenoids and flavanoids are the main chemical constituents of A.paniculata and these compounds are believed to be responsible for the biological activities of the plant (Tang et.al., 1992; Saxena et.al., 1998). It is widely used in Chinese and Ayurvedic medicine for the treatment of gastric disorders, infectious diseases and common colds. It has multiple pharmacological properties such as antiprotozoal, hepatoprotective, anti-HIV, anti-inflammatory (Sheeza et.al, 2006) antipyretic, anticancer (Li et.al., 2007), antitumor, hypoglycemic (Borhanuddin et.al., 1994), hypotensive activities and has been used for the treatment of snake bites. A primary modern use of A. paniculata is for the prevention and treatment of the common cold. It appears to have antithrombotic actions, suggesting a possible benefit in cardiovascular disease (Amroyan, 1999). Pharmacological and clinical studies suggest the potential for beneficial effects in diseases like Cancer (See, 2002; Sheeja et.al, 2007; Shi et.al., 2008; Yang et.al., 2009; Zhao et.al., 2008) and HIV infections (Calabrese, et.al. 2000). A. paniculata has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects (Bhatnagar et.al., 1961).

MATERIAL AND METHODS

Collection of plant material:-
The fresh parts of Andrographis paniculata Nees. were collected in flowering period from Amrutkund Tq, Basavkalyan, Dist. Bidar near Maharashtra-Karnataka border. The plant material were properly washed with tap water and then rinsed with distilled water.

Extraction:-
Preparation of ethanolic or methanolic or chloroform extracts:-
Fresh leaves, stem and roots of Andrographis paniculata were washed thoroughly under running tap water, shade dried and used for extraction. Dried leaves stem and roots were homogenized to a fine powder and stored in airtight...
bottles. 10gm powder of leaves; stem and roots were extracted with 100ml of each solvent (absolute alcohol, methanol and chloroform) separately for 72hr. Each parts of Andrographis paniculata extracted separately with three different solvent like absolute alcohol, methanol and chloroform. After 72 hrs of extraction, each extract was filtered through Whatman’s filter paper no.1. The filtrate was evaporated to dryness at room temperature & store at 5°C in refrigerator. Extracts were used for different tests.

Qualitative analysis:-
Extracts were tested for the presence of active principles. Following standard procedures were used (Raman, 2006; Harborne, 2005).

Test for Alkaloids:-
Ethanolic extract was warmed with 2% H2SO4 for two minutes. It is filtered and few drops of reagents were added and indicated the presence of alkaloids.
  a. Mayer’s reagent-A creamy- white colored precipitation positive.
  b. Wagner’s reagent-A reddish-brown precipitation positive.
  c. Picric Acid (1%) -A yellow precipitation positive.

Test for Steroids Terpenoid and Triterpenoids:
  a) Liebermann Burchard test: -
Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids, terpenoids and triterpenoids respectively.
  b) Salkowski Test: -
The extract was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of steroids, terpenoids and triterpenoids respectively.

Test for saponins:-
Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phenols and tannins: -
Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoids: -
A small quantity of the extracts is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the following test.
  a) Ammonium Test: -
The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonia layer. This indicates the presence of the flavonoid.
  b) Aluminum Chloride Test: -
The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted NaOH and HCl was added. A yellow solution that turns colorless indicated positive.

Test for Carbohydrate: -
Benedict's test: -
Test solution was mixed with few drops of Benedict’s reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Glycosides: -
Fehling’s test: -
Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.
Test for proteins:-
Millon’s test:-
Crude extract when mixed with 2ml of Millon’s reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for Free Amino Acids:- (Clarke, 2007)
Ninhydrin Test :-
Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Vitamin C:- (Sethi, 2003)
DNPH Test :-
Test solution was treated with Dinitrophenylhydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

For Carboxylic acid, test for NH₂, Nitrogen, Sulphur, Halogen, Amides, test for Unsaturation, test for Aromaticity
(Pratibha, 2003).

RESULT AND DISCUSSION
The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Table 1: Phytochemical screening for different extracts of *Andrographis paniculata*.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Compound</th>
<th>Test</th>
<th>Leaf extract</th>
<th>Stem extract</th>
<th>Root extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloid</td>
<td>Mayer’s reagent</td>
<td>++</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s Reagent</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Picric acid</td>
<td>++</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Amides</td>
<td>Hydrolysis with alkali</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Amines</td>
<td>Amines test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Ascorbic acid</td>
<td>DNPH test</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Carbohydrates</td>
<td>Benedict test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Carboxylic acid</td>
<td>Sodium bicarbonate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>Ammonium Test</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aluminum chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Glycosides</td>
<td>Fehling solution</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Phenol</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Proteins</td>
<td>Millon’s Reagent test</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Reducing Sugar</td>
<td>Fehling solution test</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>12.</td>
<td>Saponin</td>
<td>Frothing test</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>13.</td>
<td>Starch</td>
<td>Starch test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>Steroids</td>
<td>Liebermann - Burchard’s test</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salkowski’s Test:</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>Tannin</td>
<td>Ferric chloride test</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td>Terpenoides</td>
<td>Liebermann - Burchard’s test</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salkowski’s Test:</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>17.</td>
<td>Amino acid</td>
<td>Ninhydrin Reagent test</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>18.</td>
<td>Aromaticity</td>
<td>Flame test (Ignition test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td>Unsaturation</td>
<td>Test for Unsaturation</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

- = absent, + = Presence, ++ = Moderate, +++ = Maximum
The phytochemical analysis of *Andrographis paniculata* Nees tested were summarized in Table 1. Which revealed that presence of medicinally active compound in plant Leaf, stem and roots? For extraction of phytochemicals, ethanolic, methanolic and chloroform extracts were used. Preliminary phytochemical analysis of *Andrographis paniculata* compounds show various types of chemical compounds which provide the base line for the occurrence of the medicinally active constituents like alkaloids, flavonoids, glycosides, tannins. The *A. paniculata* were rich in alkaloid, ascorbic acid, Flavonoids, glycosides, steroids, tannins, terpenoids etc. It lacks saponin. From table it is revealed that the methanolic extract of leaf stem, root of *A. paniculata* shows maximum Phytoconstituents.

CONCLUSION
In the present study callus extract showed the presence of bioactive compound such as alkaloids, flavonoids, terpenoids, ascorbic acid, tannin, glycosides, proteins, triterpenoids, starch, phenol, etc. This study also leads to the further research in the way of isolation and identification of the active compound from the *A. paniculata* using extraction, chromatographic and spectroscopic techniques.

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REFERENCES