EVALUATION OF PHYOTOCHEMICAL CONSTITUENTS OF *ANDROGRAPHIS PANICULATA* EXTRACTS

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Abstract

Medicinal plants are an integral part of human life to treat various diseases from ancient times. The indigenous medicinal plants and plant derived drugs are an important source of alternative medicine and are extensively used to treat various health disorders. A.paniculata is one among the potential medicinal plant widely used all over the world. It exhibits various pharmacological activities such as anti-inflmmatory, anti cancer, anti–HIV, antipyretic, antidiabetic, hepatoprotective, anti bacterial and other activities. All parts of this plant are used to extract the active phytochemicals. The therapeutic value of A.paniculata is due to the presence of bioactive phytochemicals. Our present study revealed that the chloroform, methanol and aqueous extracts of A.paniclata possess various phytochemicals namely carbohydrates, glycosides, alkaloids, phytosterols, tannins, terpenoids, saponins, coumarins, proteins and amino acids. Hence, screening of bio active compounds from plants has lead to the discovery of novel therapeutic drugs which have efficient protection and treatment against various diseases.

Keywords: *Medicinal plants, Phytochemicals, Andrographis paniculata, Pharmacological activities*

1. Introduction

In the recent years, researchers are concentrating on the use of traditional medicine to treat human ailments has been revived all over the world. Medicinal plants used in traditional systems possess immense potentials. There is growing interest in correlating the phytochemical constituents of medicinal plants with its pharmacological activity (Prachayasittikul *et al.*, 2008). The various phytochemical compounds detected are known to have potential utility value in medical sciences. The Indian pharmacopoeia literature indicates that *A.paniculata* is a predominant constituent of at least 26 Ayurvedic formulations (Iruretagoyena *et al.*, 2005).

A.paniculata is commonly known as 'King of Bitter'. It is a small, annual, branched and erect plant. It grows abundantly in south eastern Asia including India, Srilanka, Java, Pakistan Indonesia and Malaysia. It prefers to grow well in a diversity of habitats such as moist, shady areas, hill slopes, plains, farms, seashores, waste lands and dry or wet lands (Prajapati, 2003). *A.paniculata* is used to treat body heat, prevent common cold, dispel toxins from body, upper respiratory tract infection, antidote against snake and insect poisons, colic pain, loss of appetite, irregular stools and diarrhea (Saxena *et al.*, 1998; Gabrielian *et al.*, 2002; Samy *et al.*, 2008). *A.paniculata* contains diterpenes, flavonoids and lactones. Flavonoids mainly present in roots and have also been isolated from leaves. Alkanes, aldehydes and ketones are existing in the aerial part of the plant (Chang and But, 1987). Hence, in the present study, the qualitative phytochemical analysis of three extracts (chloroform, methanol and aqueous extracts) of *A.paniculata* was evaluated.

2. Methodology

2.1. Collection of *Andrographis paniculata*

The experimental plant species, *Andrographis paniculata* was purchased from the local herbal market. The plant was authenticated and the voucher specimen (Specimen No. JACZOO IM1) was deposited in the herbarium of PG & Research Centre of Zoology. Jayaraj Annapackiam College for Women (A), Periyakulam, South India.

2.2. Preparation of plant extract

Fresh *A. paniculata* plants were washed thoroughly in tap water followed by distilled water and then shade dried until all the water content lost completely. Dried plants were crushed and powdered using blender. Fine powder was obtained after sieving and stored in airtight container until further use. The plant powder was extracted with three different solvents with an increasing polarity (Chloroform, methanol and aqueous solution). The successive extraction was done by a cold maceration process for seven days with regular agitation (Cooper and Gunn 2005; Singh *et al.*, 2007). After seven days of cold maceration process it was filtered through sterile muslin cloth and the solvent was evaporated using soxhlet apparatus. The residues obtained after evaporation were stored at –20°C until used for experimentation.

3. Qualitative chemical evaluation

The extract and fractions obtained were subjected to qualitative tests for the identification of various plant constituents.

3.1 Detection of carbohydrates (Kokate *et al.*, 1999)

Small amount of extract or fractions was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to Benedict's test to detect the presence of carbohydrates.

Benedict's test

Filtrate was treated with few drops of Benedict's reagent and boiled. Formation of characteristic coloured precipitate ranging from green to brick red indicates the presence of reducing sugar.

3.2 Detection of glycosides (Evans, 1989)

Small quantity of extract or fraction was stirred with 10 ml of boiling distilled water. This was filtered through Whatmann No.1 filter paper and 2 ml of the filtrate was hydrolyzed with a few drops of concentrated HC1 and the solution rendered alkaline with a few drops of ammonia solution. Five drops of

this solution was added to 2 ml of Benedict's qualitative reagent and boiled. A reddish brown precipitate showed the presence of glycosides.

3.3 Detection of proteins and free amino acids (Kokate *et al.*, 1999)

A small quantity of the extract or the fractions was dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper. The filtrate was subjected to various biochemical tests such as Millon's and Biuret tests to detect the presence of proteins and free amino acids.

Millon's Test

The extract or the fraction was treated with Millon's reagent (that contains mercuric sulphate and sulphuric acid) followed by heating gives a red precipitate or colour.

Biuret Test

To a portion of the extract or the fraction, equal volumes of 5 % sodium hydroxide and 1 % copper sulphate were added. A characteristic reddish violet colour indicates the presence of peptide linkage.

3.4 Detection of alkaloids (Mukherjee, 2002)

Small quantity of the extract or the fraction was treated with a few drops of dilute hydrochloric acid and filtered. The filtrate was treated with various reagents such as Wagner's reagent and Mayer's reagent. On treatment with dilute hydrochloric acid basic alkaloids are made soluble in water by forming salts. The alkaloids in acid solution form insoluble precipitate when treated with different reagents containing metal ions.

Wagner's Test

To, 1 ml of the filtrate few drops of Wagner's reagent that has iodine and potassium iodide was added. Formation of reddish brown precipitate indicates the presence of alkaloids.

Mayer's Test

To 1 ml of the filtrate few drops of Mayer's reagent (solution of potassiomercuric iodide solution) was added. Formation of cream coloured precipitate indicates the presence of alkaloids.

3.5 Detection of phytosterols (Klyne, 1965)

Small quantity of extract or the fraction was dissolved in 5 ml of chloroform. Then this chloroform solution was subjected to Salkowski test for the detection of phytosterols.

Salkowski Test

To 1 ml of the extract or the fraction, few drops of concentrated sulphuric acid were added along the sides of the tubes. The formation of red or pink ring is due to dehydration with concentrated sulphuric acid confirming the presence of phytosterols.

3.6 Detection of Terpenoids

To 3 ml of extract, 2 ml of chloroform and concentrated sulfuric acid were added along the sides of the tubes. The formation of reddish brown colour in the interface indicates the presence of tannins.

3.7 Detection of Tannins

To 1 ml of extract, 3 drops of ferric chloride was added. The formation of brownish green colouration indicates the presence of tannins.

3.8 Detection of saponins ((Kokate *et al.*, 1999)

The extract or the fraction was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. Steroidal glycosides (saponins) are surface active hence reduces surface tension like soap solution. The formation of foam indicates the presence of saponins.

3.9 Detection of coumarins (Dean, 1963)

To 1 ml of the extract or the fraction, 1ml of 10% NaOH was added. The formation of yellow colour is due to the cleaved products of coumarin containing phenolic groups, which form salt with sodium hydroxide.

4. Results

Table: 1 Qualitative analysis of photochemical constituents in the extract of *Andrographis paniculata*

Type of Compound	CE	ME	AE
Carbohydrates			
(i). Benedict's Test	+	+	+
Glycosides	-	+	-
Proteis & Amino acids (i). Million's Test	+	+	+
(ii). Biuret Test	+	+	+
Alkaloids (i). Wagner's Test	+	+	+
(iii). Mayer's Test	+	+	+
Phytosterols (i). Salkowski Test	-	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Saponins	-	-	+
Coumarins	+	-	+

5. Discussion

The present study on the qualitative phytochemical analysis of chloroform, methanol and aqueous extracts of *A.paniculata* exhibits the presence of carbohydrates, glycosides, tannins, alkaloids, terpenoids, saponins, coumarins, proteins and amino acids. Wiart *et al.,* (2005) reported that *A.paniculata* possesses many secondary metabolites and andrographolide is an important diterpene

lactone present in higher amount in leaves and lesser amount in seeds. Shirisha and Mastan (2013) reported the same results that the methanolic extract of whole plant of *A.paniculata* contains various types of phytochemical like fatty acids, steroids, saponins, tannis, flavonoids, anthocyanins, leucoanthocyanins, emodins, phenols and coumarins. These compounds exhibited antibacterial activity against bacterial strains such as *Staphylococcus aureus*, *E.coli*, *Pseudomonas putida* and *Bacillus subtilis*. The leaves of *Ad.vasica* have been shown the presence of phenols, flavonoids, tannins, saponins, anthraquinones, aminoacids, reducing sugars and alkaloids. Tannins are biologically active against the following microorganisms such as *E.coli*, *Staphylococcus aureus*, *Salmonella paratyphi* and *Candida albicans* (Nair and Chandra, 2004). Tannins can inhibit the growth of microorganisms at low concentration and at higher concentration it acts as an antifungal agent by coagulating the protoplasm of the microorganism (Adekunle and Ikumapayi, 2006). The phytochemical compounds are very much important for its pharmacological activity. Alkaloids have been used for the treatment of malaria, used as pain killers and managing heart diseases (Oomah, 2003). Plant steroids are important for their microbial properties and also used in nutrition, phyto medicine and cosmetics (Callow, 1936).

Flavonoids have been denoted as nature's biological response modifiers, due to their inherent ability to modify the body's reaction to allergies and virus and they exhibit their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Aiyekaagbe and Osamudiamen, 2009). Glycosides play a crucial defense role against predation by many microorganisms, insects and herbivores (De *et al.*, 1999). Phenols are a class of low molecular weight secondary metabolites present in most of the land plants. The largest group of phytochemical compound which accounts for most of the antioxidant activity in plants or plant products is phenolic compounds (Aliyu *et al.*, 2009). In intracellular histochemical staining saponin is used as mild detergent. It is also used to allow antibody access in intracellular proteins. In medicine, it is used for the treatment of hypercholesterolemia, antioxidant, anticancer, anti-inflammatory, weight loss etc and also has antifungal activity. (Lucca *et al.*, 2005). GC-MS results showed that

phenols, aromatic carboxylic acids and esters present in the chloroform extract of *A.paniculata* to be responsible for the antimicrobial activity (Roy *et al.*, 2010). Recently two new flavonoid, glycosides and a new diterpenoid (andrographic acid) were reported by Li *et al.*, (2007). The aerial parts of *A.paniculata* contain two new entlabdane diterpenoid glycosides (Zhou *et al.*, 2008) and 12 new flavonoids and 14 doterpenoids heve been reported from aerial part (Chen *et al*, 2006,). In leaves a diterpene glucoside has been detected (Weiming *et al*, 1982). The phytochemical analysis of ethanol extract of the leaves of *A.paniculata* yielded one new diterpene (13R, 14R) 3, 13, 14, 19-tetrahydroxy-ent-labda-8(17), 11-dien-16, 15-olide1 (Xu *et al.*, 2010). Chemical evaluation of methanol, hexane and chloroform extracts of root and stem of *A.paniculata* identified three flavones namely 5-hydroxy-7,8,2'-trimethoxyflavone 1,5-hydroxy-7,8-dimethoxyflavone and 5-hydroxy-7,8,2',5'-tetramethoxyflavone 3 (Radhika *et al.*, 2010). More over four xanthones were isolated from the roots of *A.paniculata* by Dua *et al.*, (2009). Our results confer the bioactive phytochemical compounds from *A.paniculata* have greater potential application either in the form of powder, extracts or in its isolated compounds in developing new therapeutic drugs.

6. Conclusion

A.paniculata shows huge variety of pharmacological activities either in the form of powder, crude extract or in its isolated compounds. Several products fortified with extract and isolated compounds have been launched in national and international markets for various diseases. *A.panicualta* could be useful as highly applied therapeutic agent for a variety of disorders in the near future to cure human diseases as well as some animal diseases.

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7. References

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